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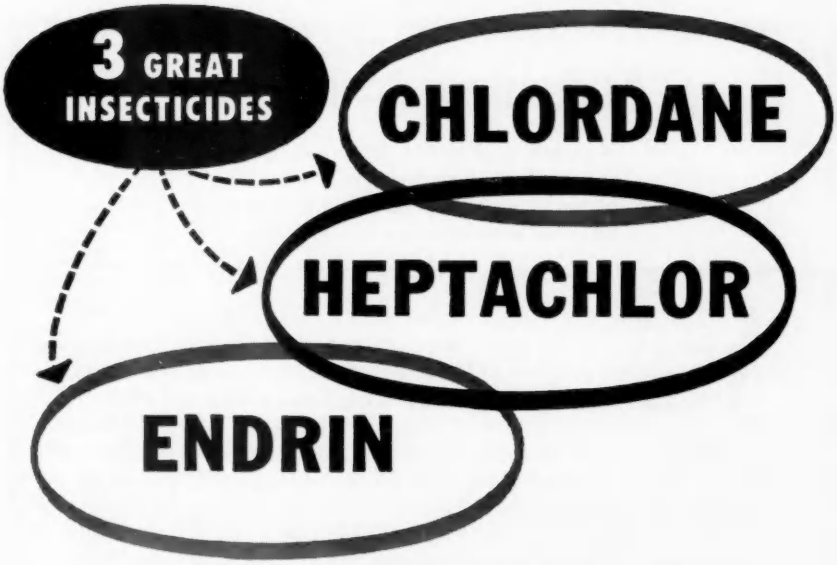

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1. Martin, G. A., Garfinkel, B. T., Brooke, M. M., Weinstein, P. P., and Frye, W. W.: *J.A.M.A.*, 151:1055, Mar. 28, 1953.
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3. Information Please: *GP*, 4:91, Sept., 1951.

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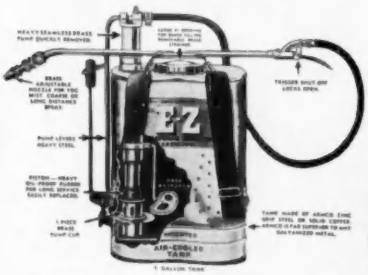

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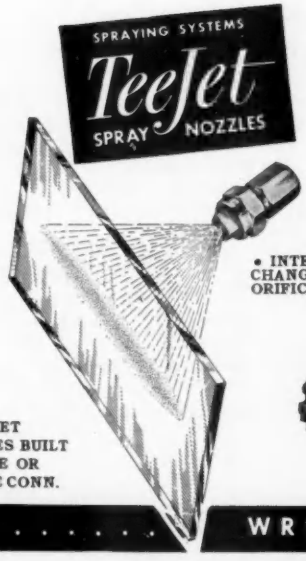

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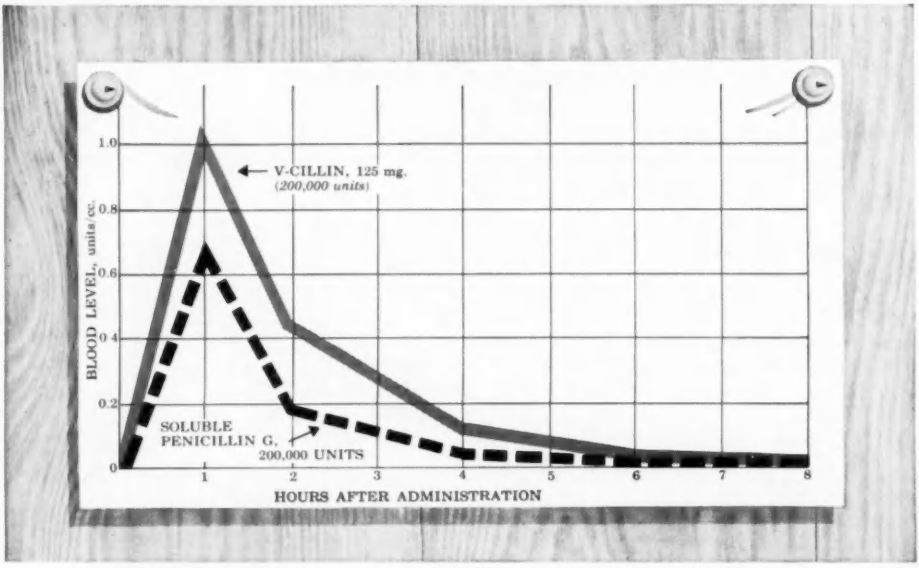

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A STUDY OF THE ECOLOGY OF WEST NILE VIRUS IN EGYPT[1]

R. M. TAYLOR[2], T. H. WORK, H. S. HURLBUT AND FARAG RIZK

Departments of Virology and Entomology, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt

For more than three years the Department of Virology of NAMRU-3 has been engaged in the investigation of the epidemiology of human infections with West Nile virus and the natural history of the virus in Egypt. Since 1953 several reports dealing with specific phases of the study have been issued (Taylor and Hurlbut, 1953; Taylor, 1953; Smithburn *et al.*, 1954; Work *et al.*, 1953 & 1955; Hurlbut, 1956; Hurlbut and Weitz, 1956). While many gaps in our knowledge need to be filled before the intricate behavior of West Nile virus, even in this environment, can be described with assurance, it is felt that enough information has been accumulated to justify a coordinated summation.

The history of West Nile virus has been reviewed in two previous communications from this department (Taylor, 1953; Work *et al.*, 1955). It will be sufficient to recall here that the virus was first discovered in the blood of a native woman of the West Nile province of Uganda, who at the time was suffering from a mild febrile illness (Smithburn *et al.*, 1940). Subsequent serological surveys revealed neutralizing antibodies against the virus to be widely disseminated in the native populations of Uganda, Kenya, the Belgian Congo and the Sudan (Smithburn and Jacobs, 1952; Smithburn, 1952).

Philip and Smadel (1943) experimentally demonstrated that *Aedes albopictus* would transmit West Nile virus, and Kitaoka (1950) made a similar observation in respect to *Culex pipiens* and *Culux tritaeniorhynchus*. The virus again came to attention when in the summer of 1950 Dr. John R. Paul collected a series of bloods from children in the Sindbis sanitary district, some thirty kilometers north of Cairo, for the purpose of examining for antibodies against the poliomyelitis viruses. In the process of these examinations West Nile virus was found in the blood of three of 250 children (Melnick, Paul *et al.*, 1951). These authors also found that a high percentage of the village population above the age of two to four years possessed neutralizing and complement-fixing antibodies for West Nile virus. This in brief was the state of knowledge concerning the ecology of West Nile virus when a program for the study of viral and rickettsial infections in Egypt was initiated by NAMRU-3 in the fall of 1951.

[1] This study was conducted under the auspices of the Ministry of Public Health of the Egyptian Government and the U. S. Naval Medical Research Unit No. 3, with aid from The Rockefeller Foundation, and latterly from the Office of Naval Research through a contract administered by the University of Chicago.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

[2] Present address: Yale University Medical School, 333 Cedar St., New Haven, Connecticut.

The prevalence of West Nile infection among inhabitants of villages situated near Cairo and the lack of information on the manner of transmission and the general ecology of the virus offered an enticing problem for investigation. During the succeeding three years, the study of the behavior of West Nile virus in this environment received major attention.

Plan and scope of the study. The object of the study was to elucidate the epidemiology of human infection and the natural history and life cycle of the virus in the climatic and biological environment of Egypt. The methods used incorporated attempts to isolate the virus from naturally infected hosts and vectors, to determine the occurrence and incidence of infection in man and other vertebrates by serological surveys for specific antibodies against the virus, and to bolster and supplement information gained from the examination of field material by laboratory experiments on susceptibility to infection of suspected hosts and the transmission of the virus by arthropods. In addition, observations were made on the prevalence and seasonal variation of the arthropod and vertebrate fauna likely to be involved in the cycle and maintenance of the virus.

Initially, human sera were collected at various points along the Egyptian Nile and examined for complement-fixing (CF) and neutralizing (NT) antibodies against West Nile. This serological survey was later extended to the Sudan and additional sampling was made in the Nile Delta in order to secure a more comprehensive concept of the geographical distribution of West Nile virus infection than that given by the previous surveys of Melnick and Paul (1951) in Egypt and Smithburn (1952) in the Sudan.

The more intensive studies were carried out in a restricted area in the upper Nile Delta where evidence pointed to a yearly recurrence or a high endemicity of the infection. It was from here that information on the age, sex, and seasonal incidence of infection in the human population was obtained; from this area also most of the bloods from birds and lower mammals, and the arthropods, particularly mosquitoes, were collected.

The development of leads strongly influenced the course and emphasis of the investigation. For example, the isolation of the virus from mosquitoes and later from birds focused attention upon them as natural vectors and hosts, and the finding that human infection was highly endemic in certain zones in the Nile Delta but not in others led to a search for the cause of this phenomenon.

There will first be recorded information gathered on the infection in man, other vertebrates, arthropod vectors, zonal activity of the virus in the Nile Delta, and data on over-wintering of the virus in an endemic area. This information will be rather tersely presented, with limited comments. Discussion and deductions will be reserved until the end, because many of the data are interrelated and their interpretations are interdependent.

METHODS AND TECHNIQUES

As methods and techniques have been described in other publications (Taylor and Hurlbut, 1953; Work *et al.*, 1953, 1955; Hurlbut, 1956), only the salient features will be given here.

Collection and handling of blood specimens. The blood specimens from man and the larger mammals were collected in vacutainers of 20 ml. capacity. For birds and small animals, syringes were employed. Techniques for obtaining blood by heart puncture from birds have been described by Work *et al.* (1955). Domestic fowl were bled from the wing vein. If the specimens were to be used for virus isolation or if there was delay beyond a few hours before they reached the laboratory, the tubes containing the blood were placed in a thermos jug with ice.

Upon arrival at the laboratory the serum was separated from the clot and transferred to small screw-cap vials and stored at −15 to −20°C. The sera to be examined for virus were inoculated immediately without previous freezing. The frozen sera were preserved for serological tests and for reinoculation in the event a viral agent was obtained from inoculation of the fresh specimen.

Collection and handling of arthropod specimens. Mosquitoes were, for the most part, collected in a New Jersey type light trap, but some captures were made in animal-baited traps, by hand capture, and by knock-down sprays in houses (Taylor and Hurlbut, 1953; Hurlbut and Weitz, 1956). Other arthropods were usually collected from their respective hosts, although most of the ticks were obtained from their favored habitat during the non-feeding period. Upon arrival at the laboratory the arthropods were classified, usually as to species, and those to be examined for virus were sent to the virus laboratory for inoculation.

Virus isolation: vertebrates. Attempts to isolate West Nile virus from vertebrates were confined largely to the blood serum. In a few instances the brain and spleen from sick birds were examined. Bloods to be examined for virus were brought to the laboratory within a few hours, never exceeding ten hours, and in the meantime were kept in a thermos containing ice. Upon arrival at the laboratory the serum was separated from the clot, cleared by centrifugation for 15 minutes at 2,000 rpm., and 0.05 ml. inoculated intracerebrally (i.c.) and subcutaneously (s.c.) into each of seven mice one to three days of age. This was accomplished by inserting a 26-gauge needle attached to a ¼ ml. tuberculin syringe under the skin between the shoulder blades, passing it upward into the occiput and injecting 0.02 ml. into the brain; then withdrawing the needle from the skull and injecting 0.03 ml. under the skin before complete withdrawal. The mice were observed daily for three to four weeks and if any died or became definitely ill or paralyzed, the brain was removed and a 10 per cent suspension prepared for passage to a new group of mice. Either 10 per cent inactivated normal rabbit serum or 0.5 per cent bovine albumin in .85 per cent NaCl buffered at pH 7.3 was used for diluent. All passage material was cultured for bacteria by streaking on blood agar plates.

If brain to brain passage of bacteria-free suspensions consistently produced paralysis and death of the mice, the presence of a viral agent was suspected. The final identification of West Nile virus was made by titration against a known West Nile immune serum.

Initially the material being examined for virus was inoculated into both infant and adult mice, but after experience demonstrated that in no instance was West Nile or any other virus recovered from adults that was not also re-

covered from infant mice, the inoculation of adult mice with the original specimen was discontinued. If, on the other hand, only adult mice had been used, many strains of viruses other than West Nile would have been missed.

In order to determine the relative sensitivity of infant and young adult mice inoculated i.c. and embryonated hens' eggs inoculated into the yolk sac, in detecting the presence of West Nile virus, the following experiment was conducted. Six human sera from which West Nile virus had been isolated were titrated in tenfold dilutions in infant mice, young adult mice and seven-day-old embryonated eggs. The mice were inoculated i.c. and the embryos inoculated into the yolk sac. The titer was consistently highest in infant mice (average 1.9 logs), next highest in adult mice (average 1.2 logs) and lowest in embryonated eggs (less than 1 log). Indeed, from one serum the virus was not recovered by egg inoculation. These sera had been preserved for several months at −20°C. and it is not improbable that fresh sera might have given higher titers. Nevertheless the titrations were made simultaneously and are indicative of the relative effectiveness of the three methods in detecting low concentrations of West Nile virus.

Yolk sac inoculation of the egg embryos was chosen because it is a simple procedure and previous experience had shown that infection with the prototype West Nile strain consistently produced death of the embryos inoculated by this route (Taylor, 1952).

During the first year and a half of the study the inoculated mice were held for four weeks before discarding, but after it was found that some if not all of the mice in the groups from which virus was recovered died or developed paralysis within three weeks, the period of observation was reduced to 21 days.

When organs (brain and spleen) were examined for virus, a 10 per cent suspension was prepared and inoculated as above described for the blood serum.

Virus isolation: arthropods. Arthropods to be examined for virus were pooled according to taxonomic classification, source and place of collection. Each pool was triturated in a porcelain mortar with pestle, suspended in the diluent (10 per cent inactivated rabbit serum or 0.5 per cent bovine albumin), centrifuged for 15 minutes at 2,000 rpm. and the supernate used for inoculation. No antibiotic was used in the initial inoculation but if the mice died from bacterial infection, the specimen was reinoculated after the addition of penicillin and streptomycin. Depending upon the requirements to control bacterial infection, as much as 2,000 units of penicillin and 10 mg. of streptomycin per 1 ml. of the arthropod suspension was added. The amount of the diluent used for suspending the arthropods varied with the size and number of the arthropods in the pool; for mosquitoes the quantity of the diluent ranged from 0.05 ml. to 0.2 ml. per insect. Only non-blooded mosquitoes were inoculated.

All specimens including both blood sera and arthropod suspensions that yielded virus were reinoculated to verify the isolation. Pending reinoculation the suspensions were retained in a cold box at approximately −20°C.

Complement fixation (CF) test. Antigen. Antigen was prepared by the benzine extraction method (De Boer and Cox, 1947). Five- to seven-day-old mice were

inoculated i.c. with a 10^{-3} dilution of West Nile virus, and with the onset of paralysis (third to fourth day), the brains were harvested by aspiration into a container immersed in a CO_2 and alcohol bath (Strome, 1953).

The brains thus collected were transferred to a small type Waring blender, to which four volumes of sterile distilled water were added, and homogenized for a total of 90 seconds. The homogenate was then centrifuged for 30 minutes at 4,000 rpm. and the supernate removed and distributed in large (200 cc.) centrifuge bottles with rounded bottom, each bottle receiving 25 to 30 ml. The suspension was then shell-frozen and lyophilized. After complete desiccation, benzine equal to the original volume was added to the desiccate along with a few glass beads to facilitate suspension of the dried material. After 30 minutes of frequent shaking, the benzine suspension was centrifuged and the supernatant benzine removed. This process was repeated twice. After the third extraction the supernatant benzine was removed and the precipitate freed of benzine by placing the bottles in a vacuum jar with an oil trap and applying moderate vacuum. To the desiccate was then added 0.85 NaCl solution equal to the volume of the original suspension, and the bottles were shaken by a shaking machine for 30 minutes and placed in the +4 ice box overnight. The following morning the suspension was centrifuged for 30 minutes at 10,000 rpm. and the withdrawn supernate constituted the antigen. For inactivation of the virus 0.05 per cent formalin was added.

To determine the potency of the antigen, it was titrated against a known West Nile immune serum. Only those antigens were used which gave fixation in dilutions of 1/16 and above. Two to four fixing units were used in the test.

Hemolytic system. The usual sheep cell, rabbit amboceptor, guinea pig complement system was used. All were prepared in the laboratory. The sheep cells were preserved, not longer than one month, in modified Alsever's solution (Kendrick *et al.*, 1945). A solution of 8.5 NaCl, 0.1 $MgSO_4$ and 0.015 grams of $CaCl_2$ (anhydrous) per 1,000 ml. of distilled water, and buffered with 0.575 barbital (acid diethylbarturic) and 0.375 grams of sodium barbital was used as diluent for all ingredients employed in the test. Twofold dilutions of the test sera commencing at a dilution of 1/4 were made. Each element in the test was contained in 0.2 ml. volume, thus making a total of 1 ml. in each tube. Two units of complement were used in the test and the primary incubation was carried out at +4°C for 18 hours. After addition of the sensitized cells, the racks were placed in a water bath at 37°C., and reading made at the end of 30 minutes.

Besides the usual anticomplementary control, normal mouse brain treated in the same way as in the preparation of the antigen was added to a tube containing the lowest serum dilution. This was done because it was found that occasionally a serum would give fixation in low dilution with normal mouse brain even after benzine extraction.

Neutralization test (NT). Neutralization tests were performed in the usual manner by mixing equal volumes of uninactivated serum with a virus dilution calculated to give approximately 100 LD_{50}. The serum virus mixture was incubated at 37°C. for two hours and 0.03 ml. inoculated i.c. into each of a group of

six mice. Survival ratios of 4/5, 5/5, 5/6, and 6/6 were recorded as positive neutralization, while a 0/5, 1/5, 0/6 or 1/6 survival ratio was taken as a negative result. Survival ratios in-between were tentatively recorded as inconclusive and the test repeated. If on retesting the result was again inconclusive, the test was so recorded.

Either 10 per cent inactivated normal rabbit serum or 0.5 per cent bovine albumin in buffered saline was used for diluent. Approximately the same titration was obtained with both diluents. It should be cautioned, however, that it is important to heat the rabbit serum for ½ hour at 56°C. before use; some presumably normal rabbits have been encountered whose unheated serum partially inactivated the virus.

RESULTS OF FIELD AND LABORATORY STUDIES

Vertebrate hosts. Information on vertebrate hosts was gained mainly through serological surveys for specific antibodies, and to a lesser degree through isolation of the virus from the blood. Also, supporting evidence on suspected hosts was sought by experimental infection. Trial infection, particularly by the means which probably apply in nature, is of value not only in verifying the specificity of the immune response but in distinguishing between hosts which play an active part in the cycle of the virus and those which do not. That is, an "active" host should circulate virus in adequate concentration to infect the arthropod vector; otherwise the virus is trapped. Such infections have been termed "dead-end" infections. These dead-end infections may, however, elicit specific antibodies to the virus. Consequently, the finding of antibodies to the virus in a species of animal does not necessarily imply that the species is involved in the cyclic propagation of the virus.

Vertebrate hosts will be dealt with under the following headings: man, other mammals, and avian species.

MAN

Susceptibility to infection. That man is susceptible to infection with West Nile virus has been amply demonstrated by the isolation of the virus from infections acquired in nature (Smithburn *et al.*, 1940; Melnick, Paul *et al.*, 1951; Bernkopf *et al.*, 1953; Taylor, 1953; and Goldblum *et al.*, 1954), by accidental laboratory infection (Hamilton and Taylor, 1954), by the frequency of neutralizing and complement-fixing antibodies among indigenous populations in an endemic environment, and by experimental infection (Southam and Moore, 1954b). Using a low mouse passage strain of Egyptian origin, Southam and Moore found that the virus reached titers in the blood that should be sufficient to infect mosquitoes. It may, therefore, be concluded that man is not only susceptible to the infection but may serve as an active host.

Distribution of West Nile antibodies in the human population of Egypt and the Sudan. Human blood collections were made at various points along the Nile Valley in Egypt and the Sudan with the object of securing information on the geographical distribution of West Nile and other viral and rickettsial infections as revealed by the presence of humoral antibodies.

Usually an effort was made to obtain blood samples from both children and adults in about equal proportion, but for practical and uncontrollable reasons it was not always feasible to follow a consistent pattern. In Egypt, specimens were secured from persons attending Health Centers or assembled by the Health Officer, and from school children; in the Sudan, from villagers called in by the tribal chief, school children and in some instances from dressing station and hospital patients. Consequently, the sampling was selective rather than random and the number taken in any one locality was usually insufficient to warrant more than a rough estimate of the incidence of infection as deduced from the presence of humoral antibodies. This "spot sampling" was done primarily to denote the geographical dissemination of the virus. Notwithstanding these limiting and influencing factors, certain general inferences may be drawn from the survey.

The results of the NT or CF tests with West Nile virus on sera collected from indigenous inhabitants along the Egyptian Nile and in the Sudan are shown in Table 1.[8] Examination for neutralizing antibodies was not done on samples from certain localities in the Sudan and in these instances the results of the CF reactions are given. In the table the "localities" are listed more or less from north to south and the corresponding "Map No." will identify the situation on the accompanying maps (Figures 1 and 2).

The data are tabulated according to the age of the donor, 0–14 years, 15 years and above, and all ages combined. Tabulation has not been made by sex as analysis failed to demonstrate any significant difference in the frequency of antibodies in males and females.

The first impression gained from casual inspection of the table is that antibodies to West Nile virus are widely disseminated throughout the Nile Valley and the southern Sudan, as at only one (Ezbet el Borg, Map No. 1) of the 40 localities sampled was there failure to find positive reactors. The over-all percentages of NT positives in bloods collected in Egypt are 44 for 0–14 years, 72 for 15 years and above, and 61 for all ages combined, and in the Sudan 28, 48 and 40 respectively (Table 2).

Antibodies in children are of greater interest because they are more likely to be indicative of local infection and because they give some indication of the recent presence of the virus. With this in mind a more careful perusal of Table 1 will reveal area trends, particularly in the sera collected in Egypt. It will be noted that in places situated in the northern portion of the Delta (Map Nos. 1–7) no, or only a few, children possessed West Nile antibodies; whereas further inland and to the south in the Delta (Map Nos. 10–16), the ratio of positives among children as well as adults is high (summarized in Table 2). This zonal distribution of West Nile antibodies along with other related information will be commented upon later under a separate heading.

In addition to the localities listed in the table, 16 bloods were collected from desert Bedouins in the arid region of the Sinai Peninsula and 35 from residents of the Gaza area to the north, likewise an arid region. Only one was positive by the

[8] The neutralization test on 331 of the sera included in this table was performed by Dr. K. C. Smithburn (Smithburn *et al.*, 1954).

TABLE 1

West Nile humoral antibodies in the indigenous human populations of Egypt and the Sudan

Map No.	Location	Test	0-14			15+			All ages		
			No.	+	%	No.	+	%	No.	+	%
					Egypt						
1	Ezbet el Borg	NT	19	0	0	28	0	0	47	0	0
2	Damietta	NT	18	0	0	30	5	17	48	5	10
3	Baltim	NT	16	1	6	22	12	55	38	13	34
4	Idku	NT	20	0	0	37	8	22	57	8	14
5	Ezbet Bisintawai	NT	7	0	0	46	35	76	53	35	66
6	Mit Abu Ghalib	NT	33	2	6	37	18	49	70	20	29
7	El Aziza	NT	21	0	0	6	2	33	27	2	7
8	El Hamoul	NT	21	2	10	29	23	79	50	25	50
9	Minyet el Nasr	NT	28	6	21	27	18	67	55	24	44
10	Kafr Mehallet Dawood	NT	9	4	44	24	22	92	33	26	79
11	Chenuda Farm "Ezbet"	NT	18	12	67	18	17	94	36	29	81
12	Damru	NT	23	14	61	21	21	100	44	35	80
13	Shiwa	NT	20	17	85	42	38	90	62	55	89
14	El Sherkaya	NT	22	20	91	38	38	100	60	58	97
15	Tel el Kebir	NT	14	12	78	14	14	100	28	26	93
16	Sindbis district	NT	65	41	63	63	58	92	128	99	77
17	Cairo	NT	15	4	27	92	60	65	107	64	60
18	El Ekhsas	NT	14	12	86	17	16	94	31	28	90
19	Shawashna	NT	13	4	31	14	12	86	27	16	59
20	Elwan	NT	13	9	69	17	17	100	30	26	87
21	El Motiha	NT	15	11	73	16	16	100	31	27	87
22	Nagada	NT	14	4	29	23	16	70	37	20	54
23	Armant	NT	16	9	56	25	23	92	41	32	78
24	Daraw	NT	18	16	89	9	7	78	27	23	85
					Sudan						
25	Khartoum	CF	29	9	31	49	24	49	78	33	42
26	El Nahud	CF	9	4	44	14	6	43	23	10	43
27	Abu Zabad	CF	31	11	35	7	5	71	38	16	42
28	Kasha, Salara and Tukma	CF	50	6	12	3	2	67	53	8	15
29	Kadugli	CF	26	6	23	1	0	0	27	6	22
30	El Muglad	CF	35	7	20	—			35	7	20
31	Um Dorein	CF	22	15	68	—			22	15	68
32	Talodi	CF	21	6	29	—			21	6	29
33	Malakal	NT	18	13	72	23	22	96	41	35	85
34	Mayen Mission	CF	7	4	57	4	2	50	11	6	55
35	Wau area	NT	8	5	63	25	24	96	33	29	88
36	Juba	NT	19	10	53	21	13	62	40	23	58
37	Kaguada	NT	18	3	17	21	12	57	39	15	38
38	Yei	NT	17	3	18	22	2	9	39	5	13
39	Torit and Keyala	NT	47	5	11	30	9	30	77	14	18
40	Katire and Gilo	NT	19	2	11	62	16	26	81	18	22

NT = Neutralization test; CF = Complement fixation test.
See Figures 1 and 2 for locations.

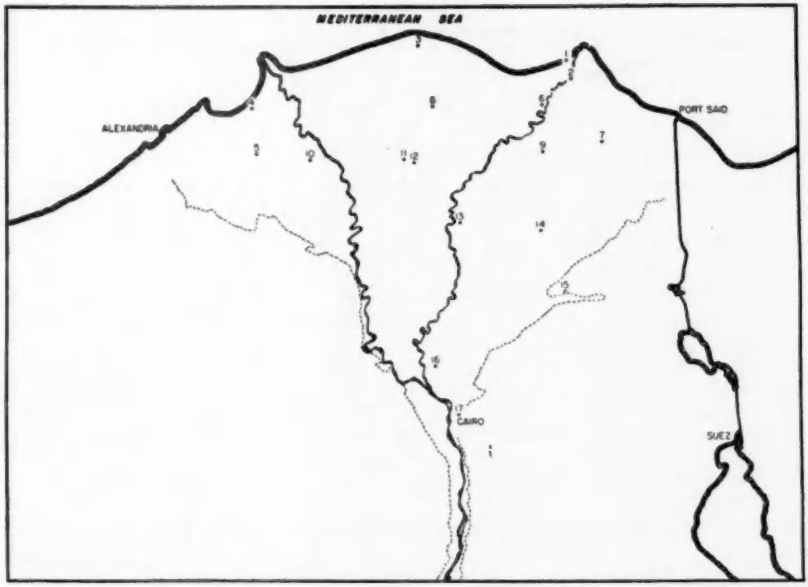

FIG. 1. Map of Nile Delta. The numbers on the map show the location of places where human blood specimens were collected. See Table 1 for results of NT or CF tests.

CF test and this came from an Arab who had resided for 15 years in the city of Suez.

On the visit to Gaza, sera were collected from lifelong residents of the area and also from refugees who had lived until five years previously in areas where West Nile virus had probably been frequently active in the past. Tests of the sera of the lifelong residents did not indicate activity of the virus in Gaza. Of the sera from former residents of a West Nile active area, 40 per cent had neturalizing antibodies against West Nile and indeed 20 per cent had CF antibodies.

Studies on human infection in the Sindbis Sanitary District. The following information on the seasonal, age, and sex incidence of human infection, as revealed by isolation of the virus and the presence of specific antibodies, was obtained from an intensive study in the Sindbis sanitary district situated about 30 kilometers north of Cairo in the Nile Delta. This area was chosen because there was reason to believe that West Nile infection was prevalent, because of its convenience to the laboratory, and because a great deal of useful demographic, sanitary, and economic information was available from a previous survey (Weir *et al.*, 1952).

Suffice it to state here that the Sindbis sanitary district incorporates five villages all within a radius of six kilometers from a health center located in the village of Sindbis. While it is purely agricultural and essentially rural in nature, nearly all of the approximately 25,000 inhabitants live in the five villages.

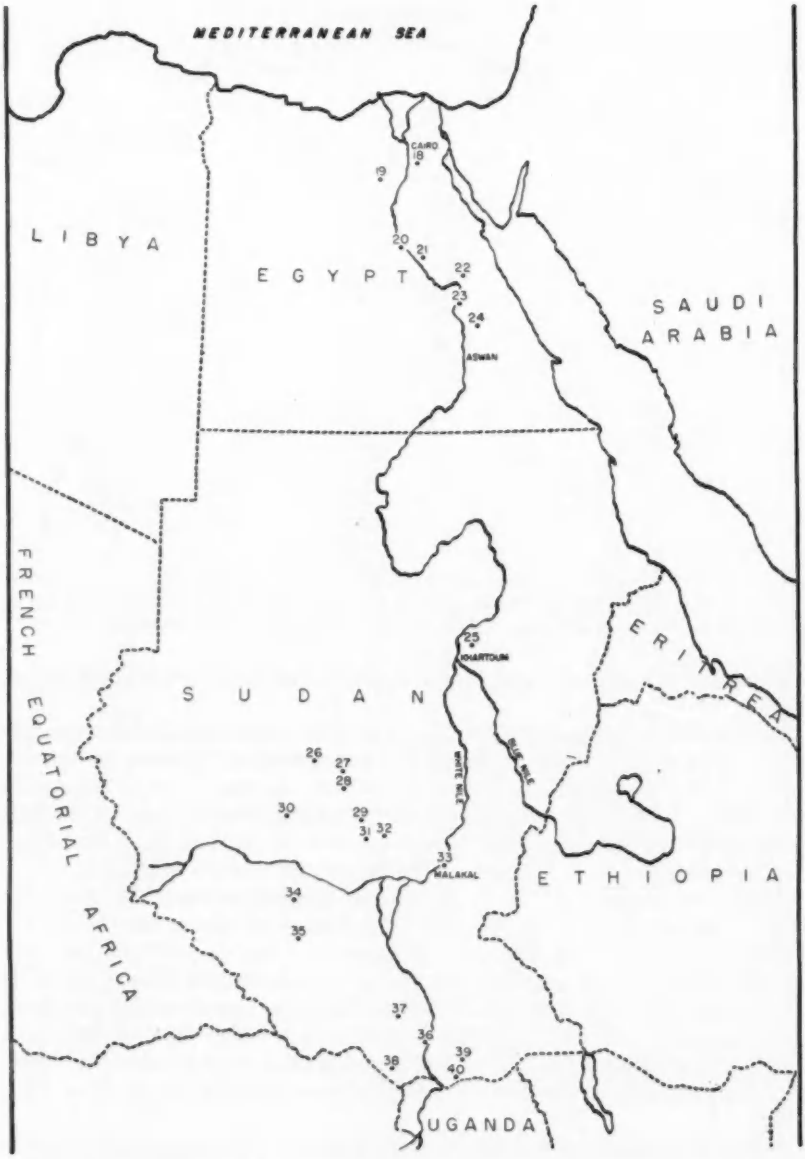

FIG. 2. Map of Egypt and the Sudan. The numbers on the map show the location of places in Egypt, with exception of the Delta, and the Sudan where human blood specimens were collected. See Table 1 for result of NT or CF tests.

TABLE 2

West Nile neutralizing antibodies in the indigenous human populations of Egypt and the Sudan summarized according to region

	Neutralization								
	0–14			15+			All ages		
	No.	+	%	No.	+	%	No.	+	%
Delta: Lower Egypt									
Non-endemic area (Map Nos. 1–7)	134	3	2	206	80	39	340	83	24
Transitional area (Map Nos. 8–9)	49	8	16	56	41	73	105	49	47
Endemic area (Map Nos. 10–16)	171	120	70	220	208	95	391	328	84
Cairo	15	4	27	92	60	65	107	64	60
Upper Egypt	103	65	63	121	107	88	224	172	77
Egypt, total	471	209	44	697	503	72	1168	712	61
Sudan	146	41	28	204	98	48	350	139	40
Grand total	617	250	41	901	601	67	1518	851	56

Note. This table is a summarization of the results of the NT reactions shown in Table 1.

Surrounding each village are about 2,000 acres of irrigated, rich, and highly cultivated land.

The houses in the villages are constructed of sun dried bricks and are closely packed, their fronts forming a continuous wall along narrow streets. Both the human inhabitants and the livestock are quartered on the ground floor but there may be a half story above for storage purposes. Aside from an occasional open space about a pond or a canal where domestic animals congregate and manure is piled or grain thrashed, there is no free ground within the confines of these villages and the over-all density of both human and domestic animal population is exceedingly high. The human inhabitants are thus not only in very intimate contact with one another but also with the numerous domestic quadrupeds—chiefly gamoose (water buffalo), cows, sheep, goats, donkeys and a few camels—and with fowl, such as chickens, ducks, pigeons and a few geese. Indeed the quadrupeds and fowl all live under the same roof with the owner and, as far as association is concerned, are virtually a part of the family.

The main roads and highways in the terrain surrounding the villages are frequently flanked with a row of casuarina, flame (royal poinciana), or eucalyptus trees, and near the villages groves of citrus fruit are not uncommon, all of which afford ample harborage for birds. The most abundant non-migratory wild birds in this district, in order of frequency are: house sparrows (*Passer domesticus*), doves (*Streptopelia senegalensis*), hooded crows (*Corvus corone sardonius*), buff-backed herons (*Bubulcus ibis ibis*), rock pigeons (*Columba livia*), and kestrels (*Falco tinnunculus*).

The study of human infection in this district consisted of attempts to isolate the virus from the blood of children taken during an acute febrile episode and to recognize previous infection by examining the blood serum for specific CF and NT antibodies for West Nile virus.

TABLE 3

West Nile virus isolations from the blood of febrile children in Sindbis District according to year

Year	No. blood specimens	Virus isolations	
		No.	%
1951–52	1009	9	0.89
1953	1302	10	0.77
1954*	513	4	0.78
Total	2824	23	0.81

* The number of bloods examined in 1954 was purposely reduced and the smaller number examined during the year does not imply that there were fewer febrile children than during the two preceding years.

Isolation of West Nile virus from human blood. The routine of obtaining blood for examination for virus was as follows. All children attending the outpatient department of the Sindbis Health Center had rectal temperatures taken by a nurse and those in a febrile state were referred to the physician for further examination. Bloods were taken from a selected number of children who according to the mothers had not been ill for more than three days, and whose physical examination revealed no specific cause of illness, such as the recognizable exanthemata, marked gastro-intestinal disturbances, pulmonary signs, or obvious bacterial or skin infections. In other words, children were selected for examination for circulating virus who were suffering from an acute undiagnosed febrile illness. Since it had been shown by serological surveys that infection with West Nile virus was likely to occur at an early age, children below four years of age were given preference in the selection.

The number of bloods thus collected and examined for virus, and the number of West Nile virus isolations, tabulated according to year are shown in Table 3. West Nile virus was isolated during each of the three years (1952, 1953, and 1954) covered by the study and from children from all of the villages during at least one year. The ratio of isolations to the number of specimens examined did not vary significantly from year to year.

In Table 4 the years are combined, but the number of specimens examined and number of virus isolations made are tabulated according to month. All of the isolations were made during the months of June, July, August, and September. The highest ratio of isolations to the number of specimens examined occurred during the months of July and August.

Table 5 shows the number of virus isolations made according to the age and sex of the children from whom the blood specimens were taken. The number of isolations in each age and sex group is too small to be statistically significant but there is a suggestion that virus is found more frequently in the blood of males than of females and in the age group of one and two years than in older and younger age groups.

West Nile complement fixing (CF) and neutralizing (NT) antibodies in human

TABLE 4

West Nile virus isolations from the blood of febrile children in Sindbis District according to month

Month	No. blood specimens	Virus isolations	
		No.	%
January	214	0	0
February	163	0	0
March	192	0	0
April	202	0	0
May	196	0	0
June	200	1	.5
July	283	12	4.2
August	270	9	3.3
September	302	1	.3
October	312	0	0
November	238	0	0
December	252	0	0
Total	2824	23	.8

TABLE 5

West Nile virus isolations from blood of febrile children in Sindbis District according to age and sex

	0–11/12 mo.		1–2 yrs.		3–4 yrs.		5–9 yrs.		10–14 yrs.		All ages	
	M	F	M	F	M	F	M	F	M	F	M	F
No. bloods	398	210	815	544	363	222	139	99	5	8	1720	1083
No. virus iso.	1		13	5	2	2	0	0	0	0	16	7
% virus iso.	0.3	0	1.6	0.9	0.6	0.9	0	0	0	0	0.9	0.6
Both sexes	608		1359		585		238		13		2803	
Virus iso.	1		18		4		0		0		23	
% virus iso.	0.2		1.3		0.7		0		0		0.8	

Note: The totals in this table differ from those in Table 4 because 21 persons whose age or sex was not definitely recorded have been omitted from this table. None of the sera from these 21 persons yielded virus.

bloods. Most of the bloods included in this survey were collected from infants and young children for the purpose of attempting virus isolation. A smaller number were also collected, however, from older children and adults solely for examining for antibodies, and the survey is therefore by no means a random sampling. It is weighted heavily in the younger age group; and moreover most of the children of this group were in a febrile state when the blood was taken. Whether or not this condition affected the results of the tests of antibodies to West Nile virus is not known. We have no reason to suspect that it did, since a subsequent survey conducted in cooperation with the WHO, in which blood samples were collected from presumably normal children, gave essentially the same pattern of CF reactors in children from 0–4 years. The data here presented include only first

bleedings. A certain number of children were re-bled but the results of these rebleedings will be tabulated subsequently.

The results of the CF and NT tests, plotted according to age of the donor, are shown in Figure 3. The striking feature is the rapid and consistent rise in both CF and NT antibodies from the first through the fourth year. By the end of the fourth year more than 50 per cent of the children gave positive reactions. The rise in those possessing NT antibodies continues with age, though less precipitously, to reach 94 per cent after the 20th year. The percentages of NT and CF positives closely parallel each other with the following exceptions: the serum of three of 17 infants below the age of six months neutralized the virus but failed to fix complement; and from the first to the third year the number of CF positives slightly exceeded the NT positives but thereafter the NT reactors were in excess. There was no significant difference in the ratio of reactors in males and females.

Further comparison of the CF and NT reactions is shown in Table 6. As might be expected the correlation of the two tests performed upon the same sera is related to the CF titer. The higher the CF titer, the closer is the correlation with the results of the NT test. There was over-all agreement between the two tests irrespective of the CF titer in 87.3 per cent of the sera examined.

All of these bloods were collected in an endemic area where the inhabitants are exposed each year, particularly during the summer months, to reinfection. In other areas where infection is sporadic or occurs only at intervals the correlation between the two tests is not as good, the ratio of CF positives tending to be lower.

It was found that the CF antibody titer was related to the age of the donor. This is shown in Figure 4. There are here included all positive CF sera reacting in a dilution of 1–4 and above, irrespective of whether an NT test was performed. It will be noted that none of five CF positive sera from infants below the age of six months reacted in a dilution above 1–4, which suggests that the reaction was due to residual maternal antibodies. This probably also applies to the three sera

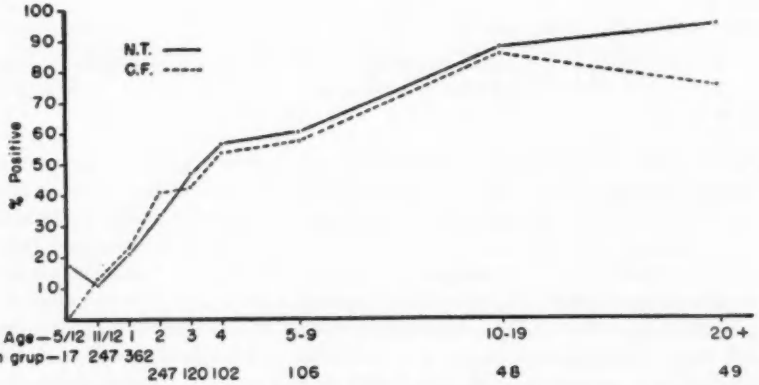

FIG. 3. Results of complement fixation (C.F.) and neutralization tests (N.T.) on sera collected in the Sindbis sanitary district plotted according to age of donor.

TABLE 6

Comparison of results of complement fixation titer and neutralization test on same sera

	Total	Complement fixation (CF)						
		Neg.	Positive				Total positives	
			1/4	1/8	1/16	1/32	#	%
Neutralization (NT)								
Neg	856	783	38	21	13	1	73	8.5
Pos	452	94	46	113	140	59	358	79.2
Total	1308	877	84	134	153	60	431	34
% pos	34.6	10.7	54.8	84.3	91.5	98.3	83.1	

Results tabulated according to "agreement" and "non-agreement" of complement fixation in dilution of 1–4 and above and neutralization of the virus

	No.	%
CF+ and NT+	358	27.4
CF− and NT−	783	59.9
Agreement	1141	87.3
CF+ and NT−	73	5.6
CF− and NT+	94	7.2
Non-agreement	167	12.8
Total	1308	100.1

of this age group that gave a positive NT reaction (Figure 3). But beyond six months of age the titers were higher in children than in adults. It is probable that this difference in the height of the titer is due not to an inherent difference in the response of children and adults but to the more recent initial infection in the children. That a drop in CF titer begins to take place within a relatively short period is implied by finding a higher ratio of reactors in dilutions above 1–16 in bloods collected from children during fall months, following the peak of virus transmission, than at any other season of the year.

Seasonal incidence of human infection. While the isolation of virus from human blood implies that infection takes place mainly during the summer season (Table 4), an effort was made to obtain more information on the seasonal incidence of infection by rebleeding, at intervals of approximately three months, those children whose blood serum on the previous bleeding contained no demonstrable West Nile antibodies. The results of retesting of these previously negative children for CF antibodies are shown in Table 7. In interpreting the results recorded in this table it should be borne in mind that a positive reaction of a blood taken during a specified month points to an infection acquired sometime during the three preceding months. Thus, a positive reaction of a blood sample taken during the month of December would indicate that infection occurred sometime during September, October, or November.

It will be noted that the highest percentages of conversions from negative to positive were found among bloods taken during the months of August through November. This would indicate that infection may have occurred anytime during the three preceding months, namely from May through October. An attempt to reckon the *probable* month of infection was made by accumulating for each month the infections that *might* have occurred during the month as revealed by subsequent conversion of a negative to a positive CF reaction. For example, infection during the month of May could have been revealed by conversions observed in blood samples taken during the months of June, July and August,

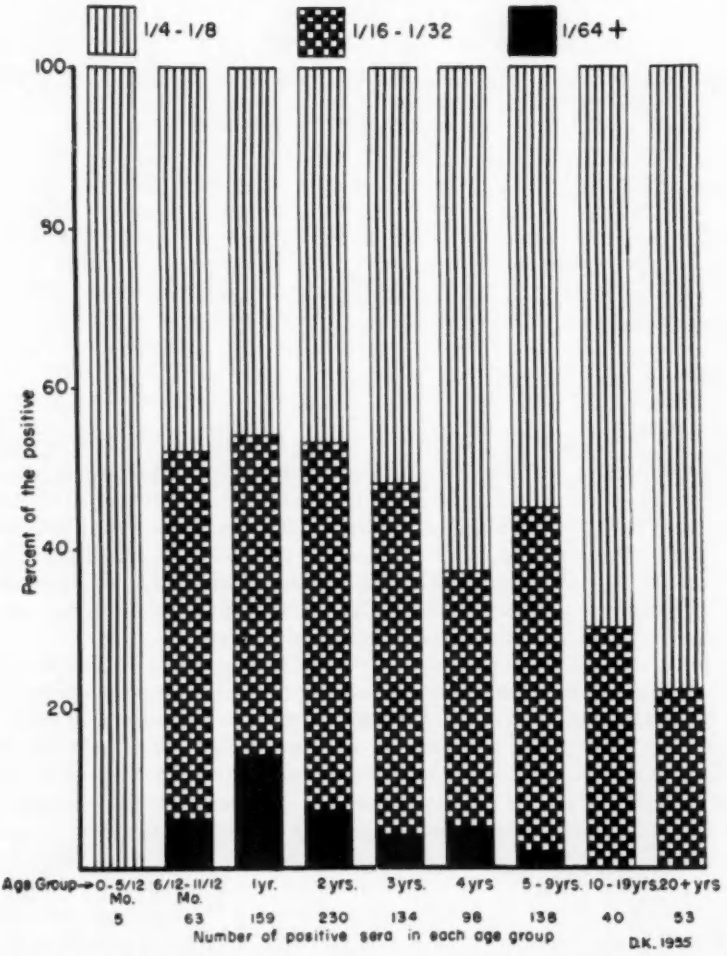

FIG. 4. Showing titer of West Nile CF antibodies in sera collected in the Sindbis sanitary district, according to age of donor.

TABLE 7

Results of retesting children at three-month intervals whose blood sera on the preceding test failed to give complement fixation with West Nile antigen

Month of rebleeding	Children tested previously neg.	C.f. results of rebleedings							
		Neg.	1/4	1/8	1/16	1/32	≥ 1/64	Total +	Pos. %
January	52	52						—	
February	87	87						—	
March	141	141						—	
April	105	103			2			2	2
May	81	78	1	1	1			3	4
June	114	114						—	—
July	118	115	2				1	3	3
August	68	54	2		3	4	5	14	20
September	136	120	1	1	6		8	16	12
October	162	123	9	4	11	8	7	39	24
November	87	73	2		2	4	6	14	16
December	177	164	2	6	4	1		13	7

TABLE 8

Showing manner of calculating monthly infection rates by adding percentage of conversions observed during the three following months

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Jan		0	0	2									2
Feb			0	2	4								6
March				2	4	0							6
April					4	0	3						7
May						0	3	20					23
June							3	20	12				35
July								20	12	24			56
Aug									12	24	16		52
Sept										24	16	7	47
Oct	0										16	7	23
Nov	0	0										7	7
Dec	0	0	0										0

Note: Since the conversion percentage observed during each month is allotted to each of the three preceding months, the figures shown in the "total" column should be divided by three to approximate the percentage of monthly infections.

for June by conversions observed during the months of July, August and September, etc.

The exact manner of calculating these monthly percentages of possible infections is shown in Table 8. As the percentage of conversions observed during a given month are allotted to the three preceding months, because infection may have taken place during any one of these three months, the effect is cumulative, and to approximate the probable infection rate for each month the percentages shown in the "total" column of Table 8 should be divided by three.

The observed monthly conversion rates (Table 7) and the calculated monthly

infection rates (Table 8, divided by three) are plotted in Figure 5. According to this method of reckoning the monthly infection rates, the great majority of infections occur during the months of May through October, with the peak in July. This substantially correlates with the period of isolation of the virus from the blood of febrile children (Table 4), except that infections calculated on the basis of CF conversions commence sooner and persist longer; this difference, however, may be due to the method of calculating the month of infection. For example, most of the infections allotted to the month of May are based on conversions observed in August, whereas it is equally if not more likely that infection occurred during the month of June or July.

The conversions observed in CF antibodies were verified by applying the NT test to a portion of these sera. The conversion pattern was essentially the same. The few infections that apparently took place during the winter and early spring months will be discussed later in considering the over-wintering of the virus.

Clinical importance of West Nile virus infection in human beings in Egypt. The importance of West Nile virus infection as a human disease problem has been a persistent question since the beginning of this study. Reports of the severe course of the infection in certain incurable cancer patients experimentally infected with extraordinarily large doses (Southam and Moore, 1952) and of widespread epidemics in Israel (Bernkopf *et al.*, 1953; Goldblum *et al.*, 1954), as well as accidental laboratory infections at NAMRU-3 have emphasized the need of obtaining reliable information on the health hazard and clinical aspects of human infections in an endemic area.

Unfortunately, this information has been difficult to secure. In the endemic area where the intensive studies were made, West Nile is essentially an infection

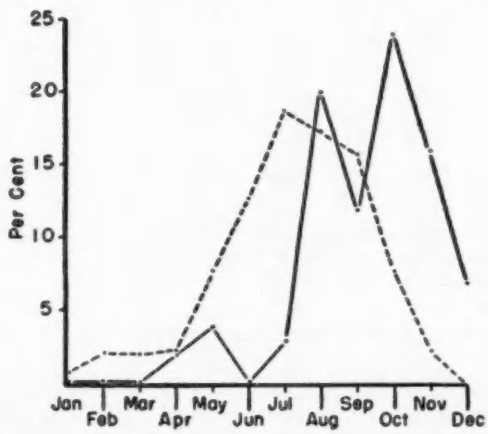

FIG. 5. Showing (a, solid line) monthly conversion percentages (Table 7) and (b, broken line) calculated monthly infection percentages (Table 8). The percentages shown in Table 8 have been divided by three to adjust for method used in calculation.

of early childhood and, according to serological conversions from negative to positive, takes place mainly during the summer months (Table 7 and Figure 5). It is also during this season that other febrile illnesses among the village children are common and widespread.

Because the diagnosis of West Nile infection can be made only by the isolation and identification of the virus from the blood of the patient or by the serological response, both of which require time, the diagnosis in an individual case is necessarily retrospective. Thus no early differentiation from other febrile disorders was possible. Although a history was taken and physical examination made when the child was first brought to the clinic and the initial blood sample taken, the child was not hospitalized and return visits were infrequent. It was therefore not possible to follow currently the course of the illnesses which later proved to be West Nile. In reviewing the records made by the physician when the child was brought to the clinic, combined with subsequent information obtained from the mothers on 22 cases proven to be West Nile by virus isolation from the blood, the following impressions were gained. Fever of rapid onset, averaging 38.5–39°C., accompanied by gastro-intestinal disturbances, malaise, profuse sweating, a fine papular rash (5 cases), moderate enlargement of the cervical, axillary and inguinal lymph nodes (3 cases), and occasionally congestion of the eyes and throat. The fever remained high for 5–6 days followed by gradual decline and a rather prolonged convalescence. No signs of central nervous system involvement were observed. As far as is known no deaths could be attributed to the infection and frank encephalitis in the highly endemic area was exceedingly rare.

The full course of the infection was followed only in two adults, both of whom were laboratory technicians. The illness of one of them has been described (Hamilton and Taylor, 1954). The course of the disease in the second technician was similar except that the onset was more sudden. Severe headache, principally in the occipital region, accompanied by muscular fatigue, particularly of the arms and legs, some initial gastric discomfort, anorexia and fever of 38 to 39°C. for several days were the outstanding manifestations. Recovery was somewhat retarded but complete.

Both of these technicians were engaged in the isolation of viruses from human blood and mosquitoes, involving the inoculation and brain-to-brain passages in mice. They were therefore exposed to the handling of freshly isolated strains. At least three other persons on the technical staff of the laboratory developed neutralizing antibodies for West Nile virus during the course of their assignment, but neither the date of the infection nor the associated symptoms, if any, were definitely determined.

It is not improbable that epidemic outbreaks of West Nile virus infection occasionally occur in the non-endemic zone along the northern fringe of the Delta but no suspect epidemics were reported during the four years covered by this study. But, as previously mentioned, febrile episodes among children are exceedingly common during the summer and an epidemic would have to be rather severe and have unusual characteristics in order to be recognized and reported.

VERTEBRATE HOSTS OTHER THAN MAN

Information on vertebrate hosts other than man was sought through three channels: a survey for specific NT antibodies in the blood serum; a search for West Nile virus in the blood and organs of animals; and experimental infection.

Survey for neutralizing antibodies. Blood sera from 882 of the more common mammal and avian species in the West Nile endemic area were examined for West Nile NT antibodies (Table 9). Noteworthy is the rather high percentage of positives among the domestic quadrupeds, with the exception of the goat, and among most avian species, especially the hooded crow and the house sparrow. None of the sera from 43 rats, five hoopoe (birds), and eight quail neutralized the virus, but varying percentages of positives were found among all others of the 24 species examined. The rebleedings of previously negative chickens refer to young chickens that were placed in a village in the Sindbis district in the summer and

TABLE 9

Results of neutralization tests on sera from domestic quadrupeds, other mammals, and avian species from endemic area

Species	Tested	Neg.	Positive	
			Number	%
Mammals:				
Camel	9	2	7	78
Cow	36	30	6	17
Donkey	15	8	7	47
Gamoose (water buffalo)	188	53	135	72
Goat	49	48	1	2
Horse	14	2	12	86
Sheep	64	49	15	23
Rattus rattus	43	43	—	—
Bats	48	44	4	8
Total mammals	466	279	187	40
Avian species:				
Chicken (domestic)	24	20	4	16
Rebleedings on previously negatives	15	13	2	13
Crow (*Corvus corone sardonius*)	163	57	102	65
Duck (domestic)	14	12	2	14
Dove (*Streptopelia senegalensis senegalensis*)	8	6	2	25
Goose (domestic)	29	23	6	27
Heron (*Bubulcus ibis ibis*)	65	47	18	28
Hoopoe (*Upupa epops major*)	5	5	—	—
Kestrel (*Falco tinnunculus*)	3	—	3	
Kite (*Milvus migrans aegyptius*)	1	—	1	
Pigeon (domestic)	59	44	15	25
Sparrow (*Passer domesticus*)	26	15	11	42
Quail (*Coturnix c. coturnix*)	8	8	—	—
Total avian species	420	250	170	40
Grand total	886	529	357	40

re-bled in the fall. Two of the 15 originally negative chickens had on rebleeding become positive, suggesting that infection occurred during their sojourn in the village.

The question naturally arises as to the specificity of the neutralization of the virus by a large proportion of the sera from domestic quadrupeds and certain avian species. Is it the result of infection with West Nile virus or some closely antigenically related virus, or is it due to the presence of a viricidal element in the serum that is unrelated to previous virus infection? We know of no generally applicable, trustworthy, and practicable method of distinguishing between specific and nonspecific neutralization or inactivation of a virus in a serum-virus mixture. But we have tried two procedures: securing and examining blood serum from animals that presumably could not have been exposed to infection with West Nile or related arthropod-borne viruses; and heating the serum at 56°C. for one half hour and reactivating with fresh normal serum.

Through the kindness of Dr. G. W. Dick, sera from 15 horses and 10 sheep were obtained from England. None of these sera neutralized West Nile virus. In contrast 12 of 14 (86%) horse and 15 of 64 (23%) sheep sera from the West Nile endemic area in Egypt were positive (Table 9). Sera from 14 horses were also obtained from Brazil, and two neutralized the virus. However, other arthropod-borne viruses antigenically related to West Nile are known to exist in Brazil, which may account for the two positive reactors.

It has been noted that non-specific viricidal action in presumably normal sera may be removed by heating to 56°C. We observed this phenomenon in some of our laboratory-reared rabbits. It was also observed that the addition of fresh normal human serum did not reactivate the heated rabbit serum although unheated serum neutralized two or more logs of the virus. Applying this criterion to 23 sera from horses, sheep, gamooses and donkeys, whose sera when originally tested was positive, it was found that the heating of these sera for one half hour at 56°C. only slightly diminished the original neutralizing action and that when mixed with fresh normal human serum the neutralization of West Nile virus was fully restored. It is felt, therefore, that such evidence as has been obtained indicates that the neutralization of West Nile virus by serum from quadrupeds collected in the West Nile endemic area is probably specific and is not due to normally present viricidal substances in the serum.

In regard to the sera from avian species, it can only be said that none of the sera from 24 chickens imported from the United States neutralized West Nile virus; and, as will be reported later, the species of wild birds that were tested demonstrated a high susceptibility to infection with West Nile virus followed by the development of NT antibodies in the surviving birds. Moreover, a few captured birds whose serum neutralized the virus were tested and found to be resistant to infection, whereas birds of the same species whose serum failed to neutralize the virus were highly susceptible to infection.

Search for virus. Specimens from 295 animals, comprised mainly of bloods from crows, herons, and domestic pigeons, were inoculated in an attempt to isolate virus, and West Nile virus was isolated twice from pigeons and once from a crow

TABLE 10

Specimens from mammal and avian species examined for virus and number of West Nile isolations

Species	No. of animals examined	Nature of specimen			WN isolations
		Blood	Brain	Spleen	
Mammals:					
Dog	3		3		
Donkey	2		2		
Horse	13	1	12		
Mule	4		4		
Total mammals	22	1	21		
Avian species:					
Chicken	4	4	4	4	
Crow (*Corvus corone sardonius*)	159	156	2	1	1
Heron (*Bubulcus ibis*)	66	61	7	6	
Pigeon	44	43	3	2	2
Total avian species	273	264	16	13	3
Grand total	295	265	37	13	3

Note: One of the isolations from pigeons was made in July and the other in August. The isolation from the crow was made in August.

(Table 10). One of the pigeons was definitely ill and the brain and the spleen as well as the blood yielded virus. Obviously in the random sampling of a bird population by means of shooting, as was done mainly with crows, the chance of obtaining a bird in the viremic stage of infection is relatively slight, although the incidence of infection among them may be rather high. Most of the herons were nestlings or were captured shortly after leaving the nest. If West Nile infection of herons during the nestling stage is frequent, the opportunity of recovering the virus from fledglings should have been greater than from older birds. No West Nile virus was obtained but several strains of another and antigenically distinct virus were isolated from the blood of these fledgling herons.

The brains of a dozen horses, four mules, two donkeys and three dogs, suspected of having central nervous system (CNS) involvement, were examined for virus during the course of these studies. Rabies virus was recovered from the brains of two of the dogs and one of the horses, but none yielded West Nile virus. However, most of the brains were either in poor condition when received or had been preserved in another laboratory for six months to two years at a temperature of approximately $-20°C$.

Susceptibility to experimental infection. In planning investigations on experimental infection of vertebrates, consideration was given to the prevalence of the vertebrate in the endemic zone, its rate of reproduction, the probable manner of infection in nature, and the height and duration of the viremia following infection.

For a vertebrate to play an important part in the continued cyclic passage and maintenance of the virus it is evident that it must be present in considerable numbers and that a yearly supply of non-immunes must be provided. It is also

evident that the vertebrate must be susceptible to infection through the prevailing natural manner, and that it must circulate virus in sufficient concentration to infect the common arthropod vector. Predominant attention was given to wild birds and certain domestic fowl because they reproduce rapidly, and because some species are numerous and the adults showed a high ratio of neutralizing antibodies against West Nile virus. A few attempts were made to infect domestic quadrupeds. As mosquitoes appeared to be the main if not the sole natural vectors of the virus, trial infection was carried out largely by permitting mosquitoes known to be infected to feed upon the experimental animal; on succeeding days the animal was bled and the blood serum titrated for virus by inoculating serial tenfold dilutions i.c. into young adult mice.

The wild birds selected for experimentation on susceptibility to infection by bite of infected *Culex* mosquitoes comprised the house sparrow, hooded crow, kestrel, buff-backed heron, and palm dove. (For a full account of these experiments consult Work, Hurlbut and Taylor, 1955.) All of the species circulated virus in varying concentration. The highest titers of circulating virus were found in the hooded crow and the house sparrow, and the lowest in the palm dove. In the house sparrow and crow the titers ranged between 3.5 and above 8 logs, and if the bird survived, the virus usually circulated for as long as six days. Though the viremia was of a lower grade in the kestrel, buff-backed heron and palm dove, some individuals circulated virus in adequate concentration to infect mosquitoes. Successful cyclic passage was tried and accomplished in all of the above enumerated species, though the percentage of mosquitoes infected following feeding was highest with the hooded crow and sparrow.

Young chicks readily acquire the infection when exposed to infected mosquitoes and circulate virus in adequate concentration to infect normal mosquitoes. In four chicks from one to eleven days of age that were infected by mosquito bite, the titer of the virus in the blood ranged from above 4 to 6.3 logs, and seven of eight mosquitoes that fed upon two of these chicks became infected. However, with the advance of age, chickens become refractory and after the third week of life are not consistently infected by mosquito bite; if virus does circulate, it is of low concentration. Thus, two of three chicks that were three weeks of age and one of eight older chickens circulated virus after exposure to infected mosquitoes, but only for one day.

The results of experimental infection of domestic pigeons were conditioned by the West Nile virus strain employed. In all of the above mentioned experiments a low mouse passage Egyptian strain isolated either from mosquitoes or from human blood was used. The mosquito strain (Ar-248) was first used in attempts to infect squabs and adult pigeons. The results were disappointing, as only one of two squabs and none of four adult pigeons circulated detectable virus after trial infection by mosquito bite, although there was a conversion to positive or inconclusive neutralization of the virus in all of the four adult pigeons one month following exposure. Somewhat better results were obtained when a West Nile strain (An-1230) isolated from a sick pigeon was used. With this strain, circulating virus titers of low order but of several days' duration were produced.

Trial infection of domestic quadrupeds was confined to two mules, two young

sheep, and a young gamoose. One of the mules received 100,000 mouse LD_{50} intravenously and the other was bitten by 15 infected mosquitoes. Virus was detected in the blood of the mule inoculated i.v. on the third day following inoculation in a dilution not exceeding 1–10. However, the blood of both mules neutralized West Nile virus one month later. Neither of the sheep circulated virus after being bitten by infected mosquitoes but one later developed neutralizing antibodies. The young gamoose was bitten by two infected mosquitoes and also failed to circulate virus. By accident the post bleeding from the gamoose was discarded before being examined for neutralizing antibodies.

Arthropod host-vectors. The term host-vectors is used because according to our present knowledge, if an arthropod is capable of active transmission of a virus, the virus is commonly harbored within the body of the arthropod for an indefinite period, usually throughout the life span of the arthropod. Thus, from the aspect of time, the designation of host is more applicable to arthropods than to vertebrates. The recognition of arthropod host-vectors of West Nile virus was attempted by search for virus in captured arthropods and by experimental infection and transmission of the virus by suspected vectors.

Search for virus in captured arthropods. Search for virus in captured arthropods was limited almost exclusively to hemophagus species. A few pools of *Musca* spp. were included because of their great abundance in Egyptian villages and the tendency of certain species to feed on secretions about the eyes and nose of children. In all, 78,067 specimens, grouped according to taxonomic classification into 1533 pools, were inoculated into infant mice in search of virus. With the exception of some of the ticks, the collections were made in or near the Sindbis sanitary district. The mosquitoes composed about two-thirds of the specimens as well as of the pools, and West Nile virus was found only in mosquitoes (Table 11).

Mosquitoes consisted of five main species: *Culex antennatus*, *Culex pipiens*, *Culex univittatus*, *Aedes caspius*, and *Anopheles pharoensis* (Table 12). Other species were so few in number that it was not deemed worth-while to examine them for virus. During 1952, taxonomic differentiation between *C. pipiens* and *C. univittatus* was not made; consequently, one column in the table is designated as either one or both of these species. However, in the two subsequent years, when the species were separated, it was found that during the season when the virus isolations were made from the undifferentiated pools, *C. pipiens* was exceedingly scarce while *C. univittatus* was abundant. It may therefore be inferred that the undifferentiated pools inoculated in 1952 were composed almost exclusively of *C. univittatus*, and that in all probability the virus was contained in this species. The fact that the percentage of isolations from the mixed pools was virtually the same as that from the pools composed exclusively of *C. univittatus*, gives further support to this inference. If this deduction is accepted, then 12 of the 17 isolations recorded were from *C. univittatus*, and five from *C. antennatus*. Relative to the number of specimens of the two species inoculated, the virus was recovered 10 times more frequently from *C. univittatus* than from *C. antennatus*, and according to the number of pools, five times more frequently (Table 12).

The virus was recovered from mosquitoes during the three successive years,

TABLE 11

Number of specimens and number of pools of arthropods examined for virus, and number of West Nile virus isolations

	No. of specimens	No. of pools inoculated	W.N. virus isolations
Fleas	3,272	54	
Flies:			
Musca spp	6,351	20	
Phlebotomus sp	123	6	
Lice	3,648	48	
Mites	6,887	80	
Mosquitoes	51,937	1003	17
Ticks:			
Amblyomma sp	94	12	
Argas spp	1,359	92	
Boophilus spp	431	16	
Dermacentor sp	133	2	
Haemaphysalis sp	12	1	
Hyalomma spp	516	42	
Ornithodoros spp	1,840	90	
Rhipocephalus spp	1,422	64	
Unclassified	42	3	
Total	78,067	1533	17

TABLE 12

West Nile virus isolations from mosquito pools according to species of mosquito

Mosquito species	Specimens	Pools	Average no. of spec./pools	W.N. virus isolations		
				Number	% of spec.	% of pools
Culex antennatus	34714	485	72	5	0.015	1.03
Culex pipiens	5514	157	35	—	—	—
Culex pipiens &/or *univittatus*	2104	53	40	3	0.14	5.66
Culex univittatus	6332	160	40	9	0.14	5.63
Aedes caspius	2027	84	24	—	—	—
Anopheles pharoensis	1246	64	20	—	—	—
Total	51937	1003	52	17		

1952, 1953 and 1954, that mosquito collections were made. The recoveries were limited to the months of July, August and September, eleven in July, five in August, and one in September (Table 13).

In Table 14 is shown the number of specimens and pools of *Culex* mosquitoes inoculated according to calendar month and the number of pools that yielded West Nile virus during the three-year period. The column headed "*C. pipiens* and/or *C. univittatus*" applies to 1952 only. While no virus was recovered until the season of the year when *C. univittatus* and *C. antennatus* become fairly plenti-

TABLE 13

West Nile virus isolations from mosquito pools according to year and month

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1952							4	3					7
1953							3	2					5
1954							4		1				5
Total							11	5	1				17

TABLE 14

Number of specimens and pools of Culex mosquitoes inoculated, and number of West Nile virus isolations, grouped according to month for the three year-period (1952–54)

	C. pipiens			*C. pipiens* and/or *C. univittatus**			*C. univittatus*			*C. antennatus*		
	No. spec.	No. pools	No. WN	No. spec.	No. pools	No. WN	No. spec.	No. pools	No. WN	No. spec.	No. pools	No. WN
Jan.	47	6										
Feb.	71	5										
March	238	7								18	1	
April	3419	46								672	13	
May	605	17		59						1999	43	
June	477	26		364			36	3		3177	66	
July	108	13		354	8	2	817	38	7	5817	83	2
Aug.	32	9		896	15	1	1019	37	1	7273	83	3
Sept.	31	4		282	12		3778	59	1	10187	121	
Oct.	59	6		137	7		635	20		4400	54	
Nov.	267	9		12	1		47	3		1087	19	
Dec.	160	9								84	2	
Totals	5514	157		2104	43	3	6332	160	9	34714	485	5

* For 1952 only. During 1953 and 1954 distinction was made between *C. pipiens* and *C. univittatus* and the two species inoculated separately.

ful, the frequency of isolation of the virus was not directly related to the abundance of these mosquitoes, since the peak of abundance of both species was not reached until September and yet all save one of the isolations were made in July and August. The highest frequency of isolations in respect to the number of specimens and pools of mosquitoes inoculated occurred during the month of July.

West Nile virus was probably recovered from mosquitoes on five additional occasions, but because the isolations could not be confirmed by subsequent reinoculation of the mosquito suspensions they have not been recorded in the tables. Three of these probable isolations were made from *C. univittatus*, one each in July, August and September. The other two consisted of one from *A. pharoensis* in July and one from *C. pipiens* in January. The one from *C. pipiens* will be referred to later, as it has a significant bearing upon the over-wintering of the virus.

Experiments on infection and transmission of the virus by arthropods. The initial

step was to introduce the virus into the hemocele by puncture or by measured injection, and observe whether the virus persisted and multiplied within the body of the arthropod. This was regarded as a screening process. If it was found that the virus was retained and increased in titer, the next step was to determine if the arthropod could transmit the virus by bite, and finally, to see if the arthropod could be infected through feeding and in turn transmit the infection to a susceptible vertebrate by bite. These experiments have been reported in detail by Hurlbut (1956). Only the pertinent features will be given here.

The screening process was applied to mosquitoes, including *A. caspius*, *C. pipiens*, *C. antennatus*, *C. univittatus*, and *Culiseta longiareolata*; house fly, *Musca domestica vicina*; soft ticks, *Ornithodoros savignyi*, *Ornithodoros erraticus* and *Argas persicus*; hard tick, *Rhipicephalus S. sanguineus*; human lice, *Pediculus humanus corporis*; mite, *Dermanyssus gallinae*; bedbug, *Cimex lectularius*; flea, *Xenopsylla cheopis*; hippoboscid fly, *Pseudolynchia canariensis*; and cockroaches, *Periplaneta americana* and *Blatella germanica*.

The virus was retained and showed evidence of growth in all the species of mosquitoes examined and also, rather surprisingly, in a wide variety of other arthropods, including house flies, human lice, the soft ticks, and the one species of hard tick. However, the titer of the virus in the mosquitoes, following a suitable period of extrinsic incubation, ranged higher than in the other arthropods. Also, it was only in mosquitoes that the active vector cycle was demonstrated, that is, infection through feeding followed by transmission through bite.

One of the soft ticks, *O. savignyi*, two weeks after being infected by injection into the hemocele, transmitted the infection by bite to infant mice, and virus was demonstrated in the coxal fluid. However, although both *O. savignyi* and *O. erraticus* acquired infection from feeding on infected mice and 27 to 33 days after the infective blood meal the titer of the virus in the body of the ticks ranged from 3.5 to 5.3 logs, neither species transmitted the infection by feeding upon normal mice.

C. univittatus held at a temperature of 28–32°C. transmitted the virus as early as five days after the infective meal, and both *C. univittatus* and *C. pipiens* were capable of transmitting the virus when kept at mean daily winter temperatures of 12–23°C.

It was found that a virus titer of 1.5 logs in the infective blood meal would infect an occasional mosquito; that a titer of 2.5 logs would infect approximately 50 per cent; and that titers of 3.5 logs and above would infect the majority if not all of the feeding mosquitoes and enable them to transmit the infection by bite.

Bionomics of mosquitoes and ticks examined for virus. Observations on the bionomics of predominant mosquitoes in the Nile Delta have been reported in the paper of Hurlbut and Weitz (1956). These studies incorporated the seasonal prevalence and the feeding habits of *C. antennatus*, *C. univittatus*, *C. pipiens*, *A. pharoensis*, and *A. caspius*.

The seasonal prevalence of the three *Culex* species, as revealed by light-trap catches in the endemic zone (Sindbis district), is graphically illustrated in Figure

6. *C. antennatus* and *C. univittatus* are most prevalent during the summer and the early fall. This likewise applies to *A. pharoensis* and *A. caspius*. On the other hand, *C. pipiens* was caught by light traps mainly during the late fall and spring. Bedroom catches from November to June were comprised exclusively of *C. pipiens* and most of the females were blooded, thus denoting that this species is active throughout the year.

Larval collections confirmed the captures of imagoes, in that *C. pipiens* larva predominated during the months of January, February and March, and October, November and December. During the warmer season, the highest percentages of larvae consisted of *C. antennatus*, *C. univittatus* and *A. pharoensis*. However, it should be noted that, although only imagoes of *C. pipiens* were found during the winter months, larvae of all of the *Culex* species as well as *A. pharoensis* and *A. caspius* were found during all seasons of the year. Thus it is probable that these mosquitoes are maintained by active breeding rather than hibernation. This was confirmed in the case of *C. pipiens* by the failure to find fat bodies associated with hibernation in any of the imagoes collected during the winter season.

Information on the feeding habits was secured from captures in traps baited with different animals, and by the precipitin reaction of the blood in the stomach of captured mosquitoes. In man-baited traps *C. pipiens* and *A. pharoensis* were caught in relatively greater numbers than in light traps, while traps baited with birds captured almost exclusively *C. univittatus*. Precipitin tests performed on blooded *Culex* mosquitoes caught in light traps, showed that 95 per cent of *C. pipiens* had fed upon man. The next most frequent feeder on man was *C. antennatus* (22 per cent), followed by *C. univittatus* (11 per cent). Avian blood was identified in 9 per cent of *C. univittatus*, 1.5 per cent of *C. antennatus*, and 0 per cent of the *C. pipiens* captured in light traps. However, a few, 0.6 per cent of *C. pipiens* captured in bedrooms during the colder months, had fed upon avian blood. Nearly all the remainder had taken blood from man (96%). It may be deduced, therefore, that *C. pipiens* is definitely anthropophilic, and that *C. univittatus* is more attracted to birds than any of the other *Culex* species. *C. antennatus* and *C. univittatus* also fed extensively on domestic quadrupeds, particularly bovids and donkeys.

All three *Culex* species extend southward along the Egyptian Nile and into the Sudan. The following information on the Sudan is supplied by Lewis (1945). *C. univittatus* is the commonest culicine in many parts of the Sudan but the typical form is believed to bite man only rarely. An atypical form (Var. *neavei*) feeds readily on man and according to Lewis is probably widely distributed along the Upper Nile (this atypical form has not been found in collections made by this laboratory in Egypt). *C. antennatus* is widely distributed throughout the Sudan and is a common man-biting mosquito. *C. pipiens* has been definitely identified only along the main Nile.

Reference to the distribution of the culicines in the lower Nile Delta and the relation to the activity of West Nile virus will be made later. The ticks were collected mainly in Egypt, but some were obtained from the Sudan and Ana-

tolia. For a review of the ecology of all the species utilized, except Anatolian *Dermacentor*, see Hoogstraal (1956).

Investigation of "endemic" and "non-endemic" zones in the Nile Delta. Allusion was made in presenting results on the extended serological survey in Egypt and the Sudan to the paucity of West Nile antibodies, particularly in children, in places sampled along the northern rim of the Nile Delta. This first came to notice in an initial survey when it was found that, in contrast to the Sindbis district, none of the bloods collected from children at Damietta, situated near the Mediterranean coast, neutralized the virus (Smithburn *et al.*, 1954). This observation was confirmed by the collection of additional blood samples in the vicinity of Damietta. It then seemed important to attempt to define the area where the virus had not invaded or was relatively inactive, from the area further south where active transmission of the virus was occurring. It was hoped that a comparison of the two environments might yield a clue to the factor or factors responsible for the maintenance and activity of the infection.

On the criterion of positive reactors among children, division may be made into two and possibly three categories. First, the localities where there are no or very few positives; this would include the localities bearing Map Nos. 1–7 in Table 1 (Mit Abu Ghalib, Map No. 6, has been included in this category because one of the two positive children had lived in a village further inland and to the south). Second, localities where the percentage of positives is slightly higher suggesting more frequent but irregular activity of the virus, Map Nos. 8 and 9. Third, localities where the percentage of positives in children is high, Map Nos. 10–16. By reference to the map (Figure 1) it will be observed that the localities falling into the first category form a crescent along the northern rim of the Delta; that the two localities in the second category are somewhat further inland; and those in the third category are distributed over the middle and southern portion of the Delta.

It was thought of interest to ascertain if these zonal differences in the ratio of West Nile antibodies in human blood also applied to crows, since this bird had been shown to be highly susceptible to infection with West Nile virus and in the Sindbis district the percentage of crows possessing specific antibodies was high. Accordingly, collections of blood from crows were made in the general vicinity of villages in the Delta where human bloods had been obtained. A tabulation of the results of neutralization tests on human and crow bloods collected in the three zones, designated as: non-endemic, transitional and endemic, follows:

Zone	Crows			Human								
				0–14			15			All ages		
	No.	+	%+	No.	+	%+	No.	+	%+	No.	+	%+
Non-endemic Map nos. 1–7	94	9	10	134	3	2	206	80	39	340	83	24
Transitional, Map nos. 8–9	64	23	36	49	8	16	56	41	73	105	49	47
Endemic Map nos. 10–16	163	106	65	171	120	70	220	208	95	391	328	84

The correlation of West Nile neutralizing antibodies in human and crow sera is evident and tends further to confirm the concept of non-endemic, transitional and endemic zones. This was referred to in the paper by Work *et al.* (1955). A high percentage of positives among crows, such as is found in the endemic zone, implies that some or many of the young crows produced each year become infected, as do the young children. The endemicity or at least the yearly activity of the virus in the Sindbis district, situated in the "endemic zone", is further attested by the isolation of the virus from mosquitoes and human blood during three successive summers. On the other hand, the absence or scarcity of positives in the non-endemic and transitional zones implies that there had been no, or only limited, activity of the virus during the life span of the crow, which probably does not average more than five to six years. This phenomenon of continuous activity of the virus in one area in the Delta and not in another was naturally intriguing, and attention was directed toward discovering the cause.

The climatic conditions in the endemic and non-endemic zones are not markedly different. As is commonly found, fluctuations in temperature near a sea coast are not so great as further inland; monthly mean minimum temperatures near the apex of the Delta (Barrage, Sindbis district) range from 2 to 3°C. lower during the winter, and monthly mean maximum temperatures 2 to 4°C. higher during the summer, than along the Mediterranean coast (Damietta). Light rains and fog are more frequent during the winter season along the lower Delta than further inland. But it is doubtful if either of these factors greatly influences the fauna and flora, since freezing temperatures are rare in either zone and water is supplied mainly by irrigation.

There are, however, rather significant differences in the density of the human population and the intensity of land cultivation. Near the coast the villages are fewer and the population ranges from 100 to 200 per square kilometer, while in the middle and southern part of the Delta the population usually exceeds 600 per square kilometer. Although agriculture is the main occupation in both zones, fishing is an industry of considerable importance in villages near the coast. The number and variety of livestock parallel the agriculture and the density of the human population. There appears to be no marked difference in the character of human dwellings and the village environment, except that at some villages near the coast livestock may be quartered outside of the village rather than under the same roof as the owner. No census of wild birds was taken but the same species were observed in both zones. Crows and other birds that feed largely on cultivated land and agricultural products may be less numerous near the coast. Yet none of these differences seemed to offer a satisfactory explanation for the absence or presence of WN virus activity.

It appeared more likely that the key might be found in vectors of the virus. As mosquitoes were the only proven vectors, emphasis was placed upon the study of this insect in the different zones. It was discovered that the total number of mosquitoes caught per trap-night was five times greater in the endemic area around Sindbis than in the non-endemic zone. It was also found that *C. univittatus,* the mosquito, from which the virus had been most frequently isolated, was

rare in the places sampled in the non-endemic zone (Hurlbut and Weitz, 1956). For example, at Mit Abu Ghalib (Map No. 6) and Bisintawai (Map No. 5) in the non-endemic zone, the average monthly catch of *C. univittatus* per trap-night from June through November was 0.018 and 0.047 respectively, while during the same period at Barada in the Sindbis district (Map No. 16) in the endemic zone, the catch was 18.06 specimens per trap-night. Moreover, no *C. univittatus* were captured at Mit Abu Ghalib and Bisintawai from July through September, the period of greatest activity of West Nile virus in the endemic zone and the time when *C. univittatus* reaches its peak of prevalence (Figure 6). However, the relation of the prevalence of *C. univittatus* to recent activity of West Nile virus, as gathered from antibodies in children and crows, is not fixed and constant; only 0.042 and 0.207 were captured per trap-night at Kafr Mahallet Dawood (Map No. 15) and Ezbet Chenuda (Map No. 11), which have been placed in the endemic zone on the basis of the serological survey. These two villages are situated along the northern edge of the endemic zone and it is conceivable that the

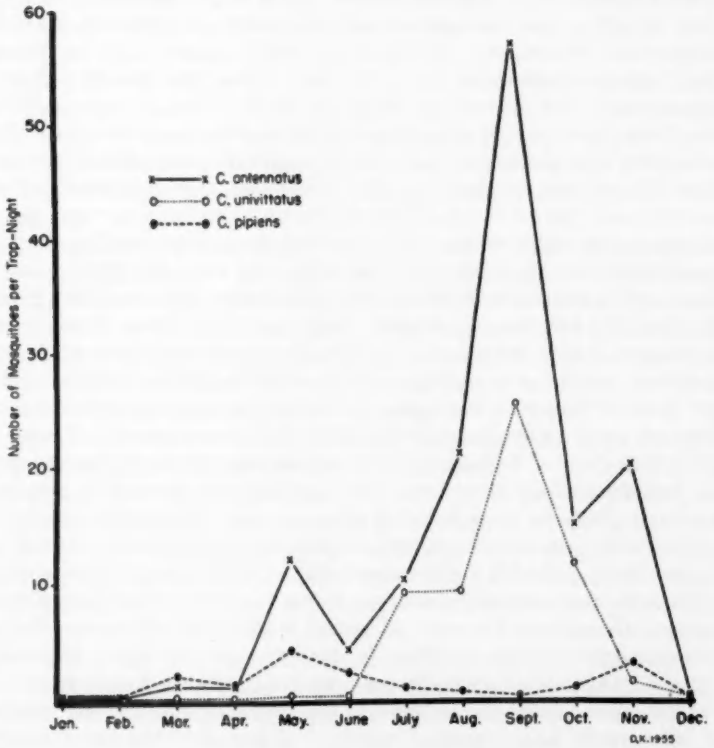

FIG. 6. Showing relative monthly prevalence of the three principal species of *Culex* mosquitoes in the Sindbis sanitary district as revealed by light-trap catches.

antibodies found there in children and in crows may have resulted from a recent invasion of the virus and are not indicative of prolonged endemicity.

Another hemophagus arthropod that has a distribution in the Nile Delta corresponding roughly to the activity of West Nile virus, is the tick *Ornithodoros erraticus* (Hoogstraal *et al.*, 1954). Yet a causal relationship is doubtful, since efforts to demonstrate transmission of West Nile virus by this tick were unsuccessful, although it may be infected by ingestion of the virus and the virus retained within the body of the tick in moderately high titer for at least thirty days (Hurlbut, 1956).

Examination of sera from British troops in the Suez Canal Zone. In collaboration with Col. M. H. P. Sayers, British Army Medical Corps, bloods were collected from 127 British soldiers who had been resident in the Suez Canal Zone from one and a half to more than two years, and from 54 persons who had recently arrived. These bloods were examined for West Nile CF antibodies. All of the 54 bloods from recent arrivals were negative and only one of the 127 bloods from resident soldiers gave a positive reaction. This blood fixed complement in a dilution of 1–32, suggesting a rather recent infection. Fifty-seven of the resident soliders were stationed near the Canal at a point separated by more than 10 miles of desert from the cultivated part of the Delta. The other 70 soldiers were stationed within two miles of the village of Tel El Kebir, situated on the edge of the Delta, where a high percentage of the native population of the village possess West Nile antibodies (Table 1), yet none of these soldiers' sera gave a positive CF reaction. It should be stated, however, that there was very little contact between the soldiers and the indigenous inhabitants and that the exercise of mosquito control about the camps kept these insects at a low level.

Investigations on over-wintering of the virus. The isolation of the virus from children and from mosquitoes during three successive summers, 1952, 1953 and 1954, the earlier isolations by Melnick, Paul *et al.* (1951) from bloods collected from children during the summer of 1950, the yearly development of specific antibodies in non-immune children, as well as the progressive increase with age of the ratio of West Nile antibodies in the human population in the Sindbis district, all point to continuous endemicity and over-wintering of the virus. Otherwise the virus would have to be introduced each summer from some distant focus, possibly by migratory birds. This possibility is discounted because the temperature gradients for a hundred miles or more up the Nile are no more favorable for the activity of mosquitoes during the winter months than those in the upper Delta. Indeed it is more than 1,000 miles to the south before climatic conditions are encountered that are conducive to uninterrupted prevalence of mosquitoes throughout the year. Moreover, water fowl are among the more important migratory birds and these in their passage rest largely in lakes near the coast. If they were responsible for introducing the virus each year, a high rate of WN antibodies in the human and the crow inhabitants in the vicinity of these lakes would be expected. Actually, it is here that the lowest antibody rates are encountered.

Having concluded that a true state of endemicity exists, three means by

which the virus might be maintained during the winter months when mosquitoes are scarce were postulated and investigated: first, in hibernating mosquitoes; second, in some long life arthropod, such as ticks; and third, by slow cyclic transmission of the virus by the relatively few mosquitoes that continue to be active.

No evidence was obtained that the three main species of *Culex* mosquitoes in Egypt hibernate during the winter months. On the contrary, consistent breeding, though retarded during certain seasons, appears to take place throughout the year (Hurlbut and Weitz, 1956).

A good many ticks of several species had been examined for West Nile virus without positive results, but after the virus was found in birds, the argasid ticks received special attention. In all 26,130 specimens, grouped into 530 pools and representing 8 different taxonomic classifications of ticks, were examined without recovering West Nile virus (Table 11). Other viruses were isolated from both *A. persicus* and *A. reflexus* ticks which were thought initially to be related to West Nile, but further study of these viruses has cast doubt on any close relationship. Moreover, attempts to infect mosquitoes with either the *A. persicus* or *A. reflexus* virus strains, which also differ from one another, have met with only limited success. These tick strains are still under study, but the present impression is that they are distinct from West Nile, and since they cannot be experimentally transmitted by mosquitoes—seemingly the main if not sole vector of West Nile virus—the theory that they may represent reversible mutants engendered by passage through different arthropod and vertebrate hosts receives little support.

Thus, no evidence was uncovered that the virus is preserved in hibernating mosquitoes or in ticks, to become active when mosquitoes become plentiful during the summer. This left the third postulate, that the virus is maintained by slow passage involving the mosquitoes that remain active during the colder months. This concept is supported by highly suggestive, if not conclusive, evidence. First, it will be recalled that a few children, two in April and three in May, converted from negative to positive reactors by the CF test (Table 7). These conversions were confirmed by the NT test. Taken at their face value, these findings signify that infection took place sometime during the preceding three months, when the mean temperature is near the minimum. Secondly, although mosquitoes are difficult to find during the winter months, *C. pipiens* does persist in an active state. As previously mentioned, they were captured in bedrooms and most of the females were blooded. Finally, West Nile virus was presumably isolated from a pool of 14 *C. pipiens* captured in bedrooms on the 27th of January. This isolation is not listed in Tables 11, 12 or 13 because it could not be confirmed by re-inoculation of the mosquito suspension three weeks later. Two mice among the seven originally inoculated died on the 7th and 11th day and passage of the brains from these two mice yielded West Nile virus. As it was recovered on a second inoculation from the brain suspension of these two mice, it must be concluded that the virus was in the mosquitoes or that contamination occurred during the initial passage.

DISCUSSION

First, it is appropriate to examine the validity of the virus isolations and the reliability of the serological reactions upon which the inferences to be drawn from these studies so largely depend.

Validity of WN virus isolations. In the handling, inoculation and sub-passage of large numbers of field specimens, cross-contamination either through human error or by some other means has been of constant concern. In spite of all reasonable precautions, it is probable that several accidental infections did occur in our laboratory. Certainly there were two or more laboratory technicians who contracted WN infection while engaged in the manipulation of freshly isolated strains. Consequently, isolations were not accepted unless confirmed by re-isolation from the original material or, in the case of isolation from human blood, by the development of specific humoral antibodies in the patient following convalescence. It is believed, therefore, that all the isolations listed in the tables are unequivocally valid. It is probable that some if not most of those that were rejected (five from human blood and five from arthropods) are also valid isolations, but, with the exception of the one from *C. pipiens* in January, 1954, their inclusion would have no significant bearing upon the interpretation of the ecology of the virus.

Reliability of serological reactions. Reasons have been given for believing that the neutralization of WN by certain mammal and bird sera is not due to some viricidal substance which may be normally present in the sera. The neutralizing action of the sera was only partially reduced by heating at 56°C. and was fully restored by the addition of fresh normal serum; the birds whose sera neutralized the virus were refractory to WN infection; and sera from horses, sheep and chickens from England and the United States exerted no neutralizing effect. Also, sera from persons who presumably had not been exposed to WN or other related arthropod-borne viruses did not contain WN antibodies, namely, British and Americans recently arrived in Egypt, and Americans used in the experiments reported by Southam and Moore (1954).

On the other hand, it is recognized that infection with one member of arthropod-borne Group B viruses (Casals and Brown, 1954) may stimulate both NT and CF antibodies against another member of the group (Smithburn, 1954; Pond *et al.*, 1955). In the initial serological survey of indigenous human inhabitants along the Egyptian Nile it was found that 12 or 8.4 per cent of the sera neutralized Japanese B encephalitis (JBE) virus and 114 or 34.9 per cent neutralized Ntaya virus (Smithburn *et al.*, 1954). Is it likely, therefore, that some of the reactions against WN are attributable to infections with these two viruses? We believe not; it seems to us more likely that the reverse is true and that infection with WN accounted for the neutralization of JBE and probably Ntaya. First of all, acute and convalescent sera from known WN infections were sent to the Rockefeller Foundation Virus Laboratories in New York, and it was there found that the convalescent sera not infrequently neutralized to varying degrees JBE and Ntaya. Indeed, Goldblum (personal communication) has reported that WN infection quite consistently stimulates neutralization of JBE. Secondly,

all of the 12 survey sera that neutralized JBE and all but five of the 114 sera that neutralized Ntaya, also neutralized WN. Finally, neither JBE or Ntaya has been recovered from any of the rather large number of arthropod and vertebrate specimens examined, and there is therefore no positive proof that either of these viruses exists in Egypt. Nor has any other virus been recovered that is closely related antigenically to WN. Thus, for lack of evidence to the contrary, it is probable that within the inherent fallibility of the test the neutralization of WN recorded in this paper is indicative of previous infection with WN virus rather than of non-specific inactivation of the virus or of infection with some other allied virus.

There is rather good correlation between the CF and NT tests on sera collected in the highly endemic Sindbis district, particularly if the sera fix complement in a dilution of 1–16 and above. The correlation varies in areas in the northern portion of the Delta which may be a reflection of the discontinuous course of the infection. It is generally supposed that CF antibodies to the viruses tend to recede and disappear with the passage of time, while NT antibodies are more permanent and may be retained throughout life. In all likelihood this applies to WN virus (Southam and Moore, 1954a), but we know of no adequate supporting data covering a sufficient length of time and involving enough individuals to be significant. It has not been possible in our studies to investigate the duration of CF antibodies, because in the area where rebleedings could be made the inhabitants were subjected, at least each summer, to reinfection, and it is conceivable that exposure to the bite of infected mosquitoes may stimulate a rise or a retention of CF antibodies which would otherwise decrease beyond detection. Indeed it is suspected that the rather high percentage of adults in the highly endemic area whose sera give a positive CF reaction may be the result of yearly exposure to infected mosquitoes. Otherwise it would be necessary to conclude that CF humoral antibodies normally persist for many years, since it is virtually certain that most of the adults in this area became initially infected during childhood. If this concept is correct it would account for the fluctuations in the ratio of CF positives to NT positives in areas where the virus is not yearly active. That is, if there had been no visitation of the virus for several years the ratio of CF positives to NT positives should be low, and if the blood samples were taken shortly following an epidemic invasion, then the ratio of CF positives to NT positives should be high, comparable to endemic areas where presumably continuous exposure to infected mosquitoes keeps the CF antibodies at a detectable level, although in the older age groups the titer is generally low (Figure 4). As only 54.8 per cent of sera which fixed complement in a dilution not exceeding 1–4 neutralized the virus, and as a few reactors in this dilution were found in areas where the NT test was negative, the specificity of the CF test in this dilution is questionable.

Granted the validity of the virus isolations and the reliability of the serological reactions, within the limits of these tests, as indicators of previous WN infection, the inferences that seem permissible on the epidemiology of the disease in man and the ecology of the virus will now be discussed.

The epidemiology of human infection. The serological surveys imply that human infection is widespread among the indigenous inhabitants of the cultivated portion of the Egyptian Nile and in the southern Sudan, but not common among desert Arabs or among British soldiers stationed in the Suez Canal zone. This distribution is consistent with the prevalence of mosquitoes. In areas not conducive to the breeding of mosquitoes or where they are artificially controlled, the infection is rare or absent. This falls in with other evidence of the mosquito's being the principal if not the only vector of the virus; in the endemic area where the intensive studies were made, the infections are highly seasonal and for the most part occur during the period when the virus is also found in mosquitoes. In this area West Nile infection is essentially a childhood disease, the adult population having acquired immunity. Most of the infections probably take place in the villages, since infected mosquitoes were repeatedly captured within the village compounds. This would account for the lack of any difference in the sex incidence of infection, because both sexes are equally exposed in the villages.

Along the coastal perimeter of the Nile Delta the infection is less frequent and is probably not endemic. It is presumed that epidemic outbreaks may occur from time to time in this region when conditions are favorable and the virus is reseeded from the interior of the Delta.

It is suspected that man is not always involved in the main channel of virus activity and that his infection may be incidental to or only partially involved in a broader cycle of the virus.

Vertebrate hosts other than man. The results of both serological surveys and experimental infection indicate that the virus is infectious to a rather wide range of vertebrates. However, as has been pointed out, a vertebrate host must circulate virus in sufficient concentration to infect the arthropod vector in order to be involved in the continuous cycle of the virus. While investigations on susceptibility to infection have not been exhaustive, the only animals found to fulfill this criterion (besides man) belong to the *Aves* class. Also their numbers and rapidity of reproduction are sufficient to furnish a continuing adequate supply of non-immune hosts. It would seem, therefore, that the main current of the virus passes through certain species of birds and young chickens with side eddies involving man, and that infection of other animals, such as the domestic quadrupeds, may be purely tangential. Experiments on susceptibility of this latter group included only mules, sheep, and one gamoose, but none circulated virus to an appreciable degree. Another reason for discounting the role of quadrupeds is that sheep, goats and gamooses are the more common species in the villages and only in sheep and gamooses were WN antibodies frequent, both of which species failed to circulate virus when bitten by infected mosquitoes.

Arthropod host-vectors. WN virus is retained and may increase in a wide variety of arthropods, including, besides mosquitoes, ticks, lice, fleas and even house flies when introduced by puncture into the body of the arthropod. But when the prerequisites for an effective vector are applied the field is narrowed to the mosquito, since only the mosquito was found capable of acquiring the infection by feeding upon an infected animal and transferring it to another animal by bite.

Thus, as far as is now known the mosquito is the only proven transmitter of the virus and only from the mosquito has the virus been recovered in nature.

Probable cycle of the virus. Naturally, the ensuing discussion on the cycle of the virus applies to the environment where these studies were made, and particularly to the endemic area centered about the Sindbis district, but the deductions may also hold for the greater part of the Nile Valley and the Sudan.

As has been pointed out, the only vertebrates and the only arthropods that have been found to fulfill the requirements of host and vector in the continuous cyclic propagation of the virus are man, birds and mosquitoes. Because the virus circulates in higher concentration in certain species of birds than in man, and because the species of mosquito (*C. univittatus*) from which the virus was most frequently recovered has an avidity for avian blood, it seems probable that the dominant cycle may be through birds and mosquitoes. This would fall in with the greater frequency of isolation of the virus from mosquitoes during the early part of the mosquito season when the crop of young non-immune birds becomes available. This combination of a plentiful supply of susceptible birds with the onset of the emergence of *C. univittatus* probably serves as a springboard for the rapid increase in the activity and dissemination of the virus in which the non-immune children become involved. This activity continues, but on a diminishing scale probably as a result of a decrease in the non-immune birds and children, through the remainder of the summer and early fall. Though children may participate in the cycle, its yearly recrudescence is probably dependent upon the spring supply of young susceptible avian species. For, considering the high immunity rate in the human population, it is difficult to conceive that the virus could be maintained by the man-mosquito cycle alone, even though the birth rate is high.

The presence of WN neutralizing antibodies in the blood of domestic quadrupeds, notably the camel, cow, gamoose and horse, indicates that they are frequently infected, but it is doubtful if they participate in the cycle of the virus; the available information suggests that if viremia does occur it does not reach a level sufficient to infect mosquitoes. If this is true, then the infection in quadrupeds is tangential and represents a dead-end type of infection, detectable only by the development of neutralizing antibodies. This concept of the cycle of the virus is graphically illustrated in Figure 7.

It is not within the scope of this paper to review the literature and discuss the relation of the postulated cycle and ecology of West Nile to other arthropod-borne viruses. But the analogy to the equine encephalitis viruses, St. Louis, Japanese B and Murray Valley encephalitis is obvious, notably in the involvement of mosquitoes as vectors and birds as vertebrate hosts. (For reviews and references see Ferguson, 1954; Anderson, 1954; Hammon, Reeves and Sather, 1951; Tigertt and Hammon, 1950.)

Over-wintering and dissemination of the virus. The only positive evidence unearthed on the over-wintering of the virus in parts of the Nile Delta pointed to a retarded but continuous passage of the virus during the three or four colder months of the year by the relatively few but active *C. pipiens* which persist through this season of the year. The development of WN antibodies in a few

children during the winter months, the presence and activity of *C. pipiens* in bedrooms, the fact that the virus will multiply and can be transmitted by mosquitoes (including *C. pipiens*) during ambient winter temperatures, not to mention the probable isolation of WN from *C. pipiens* in the month of January, all make a rather convincing case. And yet, if this is the way the virus survives the winter, its survival must hang by a slender thread, considering that most of the human population is immune and that only rarely does *C. pipiens* feed upon birds.

Though the regular and consistent transport of the virus from the tropical climate of the southern Sudan to the Nile Delta by migrating birds seems unlikely, it is highly probable that it is disseminated by birds within the home range of such indigenous non-migratory species as the hooded crow, buff-backed heron, domestic sparrow and pigeon. It is asserted that the rock pigeon changes its habitat from time to time and may therefore carry the virus from some distance. No data were collected on the flight or wind-drift range of mosquitoes. There is some travel of the human population but that of children, who are most likely to be infected, is usually limited to the neighboring villages.

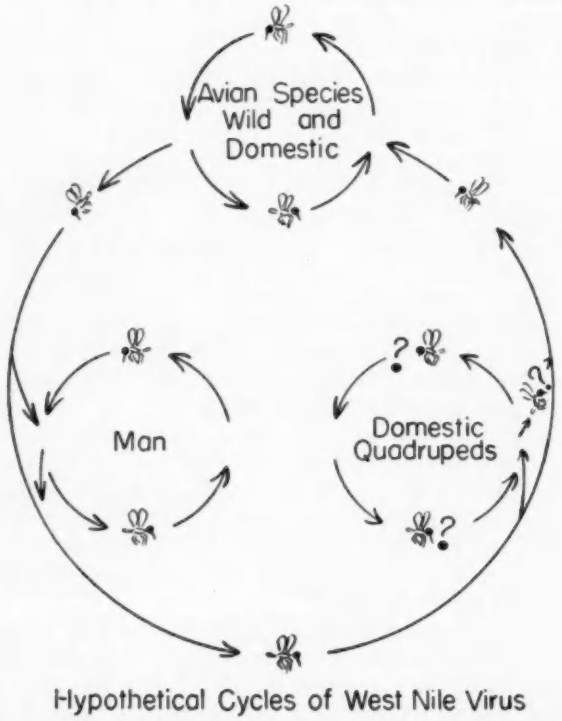

FIG. 7. Diagrammatic illustration of hypothetical cycles of West Nile virus. Note that a complete cycle involving domestic quadrupeds alone is questioned.

The above remarks apply to the Nile Delta, the southern part of which seems to provide sufficiently favorable conditions for the over-wintering of the virus and the creation of a continuous endemic state. It is not unlikely, however, that the truly migratory birds may be involved in spreading the infection along the less cultivated and sparsely inhabited portion of the Nile Valley and particularly beyond the northern boundaries of Egypt. This possibility deserves investigation.

Endemic and non-endemic zones in the Nile Delta. The results of serological surveys clearly imply that WN infection of both the human and the hooded crow population is less frequent along the northern fringe of the Delta than further inland, which led to the somewhat arbitrary division into endemic, transitional and non-endemic zones. A study of the biological environment in the endemic and non-endemic zones revealed differences which alone, or more likely in combination, may account for the variation in the persistence and activity of the virus. The human population is not so dense, the land not so intensely cultivated, and mosquitoes and apparently birds that are associated with cultivated land are not so numerous in the non-endemic as in the endemic zone. Also, and this may be a crucial factor, *C. univittatus* is relatively much less prevalent in the places sampled in the non-endemic and in the northern edge of the endemic zone than in the highly endemic area about Sindbis.

The permanent maintenance of a parasite demanding a constant supply of favorable hosts and effective vectors is frequently tenuous, and any imbalance in the essential requirements of the biological environment may break the chain in its cyclic propagation. It is believed, therefore, that a less dense human and bird population, combined with relative paucity of the most effective vector may be responsible for the failure of permanent tenure of the virus in the northern part of the Delta. However, it is surmised that this northern area is being periodically reseeded from the permanently endemic area to the south, probably by birds. Districts within the home range of non-migratory birds from the truly endemic area would be seeded more frequently, and serologically might be indistinguishable from the endemic area, while villages further removed would be subjected to less frequent invasions of the virus, manifested by a low percentage of WN antibodies among the inhabitants, especially in the younger age group.

SUMMARY AND CONCLUSIONS

The results are recorded of an investigation of the geographical distribution of West Nile virus in Egypt and the southern Sudan, the epidemiology of human infection, and the general ecology of the virus in the Nile Delta, as revealed by virus isolations, surveys for specific antibodies and study of the biological environment.

According to serological surveys the virus is widely disseminated along the Egyptian Nile and in the southern Sudan; 61 per cent of 1168 human bloods collected in Egypt and 40 per cent of 350 collected in the Sudan neutralized WN virus.

In an endemic area in the Nile Delta where the intensive studies were conducted, West Nile infection is essentially a childhood disease with yearly peaks

of transmission during mid-summer. No reliable information on the symptomatology of the infection in children was obtained, but the impression was gained that it is a self-limited, non-fatal febrile disease rarely associated with definite manifestations of encephalitis.

The virus was repeatedly isolated from the blood of febrile children (23) and from mosquitoes, *Culex univittatus* (12) and *Culex antennatus* (5), twice from pigeons (*Columba livia*) and once from the hooded crow (*Corvus corone sardonius*). The fact that *C. univittatus* was more frequently found to be infected than any other species of mosquito may be due to its relative predilection for avian blood. None of 530 pools of other arthropods, including ticks, lice, fleas, mites and flies, yielded virus.

On both experimental and ecological grounds it is concluded that the main cycle of the virus is probably through mosquitoes and birds in which man may become involved. Serological evidence points to infections of domestic quadrupeds, but these are probably only tangential or dead-end infections.

It is likely that non-migratory birds are responsible for the local dissemination of the virus but, as it appears to be endemic in parts of the Nile Delta, its transport over long distances by migratory birds was not investigated. There is evidence for believing that the virus over-winters through a process of retarded transmission by the mosquitoes that remain active throughout the colder months, although the possible role of ticks as a reservoir of the virus should be further investigated. Non-endemicity in the northern rim of the Delta may be accounted for by the less dense human and bird population there and the scarcity of what appears to be the most important vector, *C. univittatus*.

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REFERENCES

Anderson, S. G., 1954. Murray Valley encephalitis and Australian X-disease, *J. Hyg.* **52:** 447–468.

Bernkopf, H., Levine, S., and Nerson, R., 1953. Isolation of West Nile virus in Israel, *J. Inf. Dis.* **93:** 207–218.

Casals, J., and Brown, L. V., 1954. Hemagglutination with arthropod-borne viruses, *J. Exp. Med.* **99:** 429–449.

DE BOER, C. J., AND COX, H. R., 1947. Specific complement-fixing diagnostic antigens for neurotropic viral diseases, *J. Immunol.* **55:** 192–204.

FERGUSON, F. F., 1954. Biological factors in the transmission of American arthropod-borne virus encephalitis, U. S. Dept. Health & Education & Welfare. *Public Health Monograph* No. 23.

GOLDBLUM, N., STERK, V. V., AND PADERSKI, B., 1954. West Nile fever. The clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases, *Amer. J. Hyg.* **59:** 89–103.

HAMILTON, P. K., AND TAYLOR, R. M., 1954. Report of clinical case of West Nile virus infection probably acquired in the laboratory, *Am. J. Trop. Med. & Hyg.* **3:** 51–53.

HAMMON, W. McD., REEVES, W. C., AND SATHER, G. E., 1951. Japanese B encephalitis virus in the blood of experimentally inoculated birds, *Amer. J. Hyg.* **53:** 249–261.

HOOGSTRAAL, H., 1956. African Ixodoidea I. Ticks of the Sudan with special reference to Equatoria Province, U. S. Navy, Washington, D. C. (In press)

HOOGSTRAAL, H., SALAH, A. A., AND KAISER, M. N., 1954. Summary of the known distribution and biology of *Ornithodoros erraticus*, *J. Egyptian Pub. Health Assoc.* **29:** 127–138.

HURLBUT, H. S., 1956. West Nile virus infection in arthropods, *Am. J. Trop. Med. & Hyg.* **5:** 76–85.

HURLBUT, H. S., AND WEITZ, B., 1956. Some observations on the bionomics of the common mosquitoes of the Nile Delta, *Am. J. Trop. Med. & Hyg.* In press.

KENDRICK, D. B., JR., ELLIOTT, J., REICHEL, J., JR., AND VAUBEL, E. K., 1945. Supply of preserved blood to European Theater of Operations, *Bull. U. S. Army M. Dept.* (No. 84) pp. 66–73.

KITAOKA, M., 1950. Experimental transmission of the West Nile virus by the mosquito, *Jap. Med. J.* **3:** 77–81.

MELNICK, J. L., PAUL, J. R., RIORDAN, J. T., BARNETT, VoH. H., GOLDBLUM, N., AND ZABIN, E., 1951. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus, *Proc. Soc. Exper. Biol. and Med.* **77:** 661–665.

PHILIP, C. B., AND SMADEL, J. E., 1943. Transmission of West Nile virus by infected *Aedes albopictus*, *Proc. Soc. Exper. Biol. and Med.* **53:** 49–50.

POND, W. L., RUSS, S. B., ROGERS, N. G., AND SMADEL, J. E., 1955. Murray Valley encephalitis virus: its serological relationship to the Japanese—West Nile—St. Louis encephalitis group of viruses, *J. Immunol.* **75:** 78–84.

SMITHBURN, K. C., 1952. Neutralizing antibodies against certain recently isolated viruses in sera of human beings residing in East Africa, *J. Immunol.* **69:** 223–234.

SMITHBURN, K. C., 1954. Antigenic relationships among certain arthropod-borne viruses as revealed by neutralization tests, *J. Immunol.* **72:** 376–388.

SMITHBURN, K. C., HUGHES, T. P., BURKE, A. W., AND PAUL, J. H., 1940. A neurotropic virus isolated from the blood of a native of Uganda, *Am. J. Trop. Med.* **20:** 471–492.

SMITHBURN, K. C., AND JACOBS, H. R., 1942. Neutralization tests against neurotropic viruses with sera collected in Central Africa, *J. Immunol.* **44:** 9–23.

SMITHBURN, K. C., TAYLOR, R. M., RIZK, F., AND KADER, A., 1954. Immunity to certain arthropod-borne viruses among indigenous residents of Egypt, *Am. J. Trop. Med. & Hyg.* **3:** 9–18.

SOUTHAM, C. M., AND MOORE, A. E., 1952. Clinical studies of viruses as antineoplastic agents, with particular reference to Egypt 101 virus, *Cancer* **5:** 1025–1034.

SOUTHAM, C. M., AND MOORE, A. E., 1954a. Anti-virus antibody studies following induced infection of man with West Nile, Ilheus and other viruses, *J. Immunol.* **72:** 446–462.

SOUTHAM, C. M., AND MOORE, A. E., 1954b. Induced virus infections in man by Egypt isolates of West Nile virus, *Am. J. Trop. Med. & Hyg.* **3:** 19–50.

STROME, C. P. A., 1953. A closed vacuum system for harvesting infectious infant mouse-brain material, *Proc. Soc. Exper. Biol. and Med.* **84:** 287–288.

TAYLOR, R. M., 1952. Studies on certain viruses isolated in the tropics of Africa and South America: their growth and behavior in the embryonated hen egg, *J. Immunol.* **68:** 473–494.

TAYLOR, R. M., 1953. Observations on the epidemiology of West Nile virus infection. Presented at the Proceedings of the 5th International Congress of Trop. Med. & Malaria, Istanbul, 1953. NAMRU-3 Research Project No. NM 007 082.13.08.

TAYLOR, R. M., AND HURLBUT, H. S., 1953. Isolation of West Nile virus from *Culex* mosquitoes, *J. Roy. Egyptian Med. Assoc.* **36:** 199–208.

TIGERTT, W. D., AND HAMMON, W. McD., 1950. Japanese B encephalitis: a complete review of experience on Okinawa 1945–1949, *Am. J. Trop. Med.* **30:** 689.

WEIR, J. M., WASIF, I. M., HASSAN, F. R., ATTIA, S. D. M., AND KADAR, M. A., 1952. An evaluation of health and sanitation in Egyptian villages, *J. Egyptian Pub. Health Assoc.* **27:** 55–114.

WORK, T. H., HURLBUT, H. S., AND TAYLOR, R. M., 1953. Isolation of West Nile virus from hooded crow and rock pigeon in the Nile Delta, *Proc. Soc. Exper. Biol. and Med.* **84:** 719–722.

WORK, T. H., HURLBUT, H. S., AND TAYLOR, R. M., 1955. Indigenous wild birds in the Nile Delta as potential West Nile virus circulating reservoirs, *Am. J. Trop. Med. & Hyg.* **4:** 872—888.

THE ISOLATION OF ILHÉUS VIRUS FROM WILD CAUGHT FOREST MOSQUITOES IN TRINIDAD[1]

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Ilhéus encephalitis virus was originally isolated from the vicinity of Ilhéus (Bahia), Brazil by Laemmert and Hughes (1947). The agent was recovered on one occasion from a pool of *Aedes—Psorophora* mosquitoes of which the predominant species were *Aedes serratus* and *Psorophora ferox*. Successful transmission was effected in the laboratory to baby mice through the bites of *Aedes aegypti*, *Aedes serratus* and *Psorophora ferox*. Neutralizing antibodies to Ilhéus virus were also demonstrated in the sera of two rhesus monkeys, one laboratory worker and several residents of the Ilhéus area. Until the present report, Ilhéus virus had never been reisolated in nature and all work with the agent had stemmed from the original Brazilian strain.

Recent immunity surveys in Trinidad, B.W.I., have revealed the presence of neutralizing antibodies to Ilhéus virus in a high percentage of the sera collected from certain regions of the island (Downs, Anderson and Theiler, 1956). Accordingly, a study was undertaken of the epidemiological aspects of Ilhéus virus infection in man, and intensive efforts were made to isolate the virus from human beings and mosquitoes. The details of the isolation of two virus strains from mosquitoes and the identification of them as strains of Ilhéus virus are reported below.

ISOLATION

The first strain (TRVL Specimen No. 5800) was isolated from a collection of 75 mosquitoes representing eight genera (see Table 1). The mosquitoes were captured in the Melajo Forest about seven and a half miles north of Sangre Grande, Trinidad, B.W.I., on September 13, 1954. The pool of mosquitoes was ground in a mortar and suspended in 9 ml. of bovalbumin diluent.[2] The suspension was centrifuged in a 4°C. cold room for 30 minutes at about 10,000 RPM in an angle-head centrifuge. The supernatant fluid was removed and 0.03 ml. of it was inoculated, intracerebrally, into each of six 3–4-week-old Swiss mice. A portion was also stored in the dry ice box in a sealed glass ampoule. After an incubation period of from five to six days, five of the mice showed definite signs of illness. Three of the mice were sacrificed and suspensions prepared from their brains were passaged into groups of adult mice. An agent of high virulence for

[1] The studies and observations which this paper is based upon were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme and The Rockefeller Foundation.

[2] The diluent is composed of: 0.12 molar sodium chloride; 0.006 molar monobasic sodium phosphate; 0.012 dibasic sodium phosphate; and 0.75% fraction 5 bovalbumin. The pH is adjusted to between 6.8 and 7.3 by the addition of 0.3 molar sodium hydroxide solution. The diluent is sterilized by filtration through a Seitz EK pad.

TABLE 1

Composition of mosquito pools from which strains 5800 and 6851 were isolated

Strain 5800 (Melajo Forest)		*Strain 6851* (Charuma Forest)	
Mosquito species	Numbers	Mosquito species	Numbers
Psorophora feroz	22	*Psorophora feroz*	89
albipes	6	*albipes*	2
Aedes serratus	18	*cingulata*	52
scapularis	10	Total	—
hortator	2		143
fulvus	1		
Culex spissipes	2		
sp.	2		
Mansonia venezuelensis	1		
Anopheles homunculus	1		
Limatus durhami	1		
flavisetosus	3		
Trichoprosopon theobaldi	1		
Wyeomyia scotinomus	5		
Total	75		

adult mice was established and was easily maintained in serial brain to brain passage. The agent passed readily through a bacteria-tight Seitz EK pad. The virus rapidly became adapted to mice and no significant change in the 4-day incubation period was noted from the second to the sixty-eighth passage. The virus was capable of producing death of adult mice following the intraperitoneal inoculation of large doses. The virus was successfully reisolated from the sample of the original mosquito suspension stored in the dry ice box.

The second strain (TRVL Specimen No. 6851) was isolated from a collection of 143 mosquitoes representing three species of *Psorophora* (Table 1). The mosquitoes were captured at the end of the Charuma Road in the Charuma Forest, Tabaquite, Trinidad, B.W.I., on October 12, 1954. The Charuma Road site where these mosquitoes were caught is about 22 miles (crow flight) from the Melajo Forest collection site, almost in the geographical center of the island. The mosquito suspension was prepared in the same manner as the one described above, except that the total volume of bovalbumin diluent employed was 12 ml. A group of six young adult mice was inoculated intracerebrally and a sample was stored in the dry ice box. Three of the inoculated mice showed obvious signs of illness on the eighth post-inoculation day. Inoculation of normal mice intracerebrally with brain suspensions prepared from the brains of two of the sick mice reproduced the disease, and the agent was easily maintained in passage by this technique. The agent passed readily through a bacteria-tight Seitz EK pad. This agent did not adapt as readily to mice as did the first strain. The incubation period for the first few passages was six to seven days, and often one or two of the mice survived the inoculation of a ten per cent brain suspen-

sion. The incubation period gradually decreased to four days on continued passage and the virus became more uniformly lethal for the mice. The 6851 strain likewise produced a fatal infection in mice inoculated intraperitoneally with a large amount of virus. Reisolation of this agent from the original mosquito suspension has not been attempted.

IDENTIFICATION

The recent immunity survey in Trinidad revealed the presence of neutralizing antibodies to a number of viral agents in the sera of various residents of the island (Downs *et al.*, 1956). Accordingly, specific antisera against these agents were sent from The Rockefeller Foundation Virus Laboratories in New York to aid in the identification of viruses isolated in Trinidad. Immune sera to the following viruses were tested for their ability to neutralize strains 5800 and 6851: Eastern, Western and Venezuelan equine encephalitis; St. Louis; Ilhéus; yellow fever; Bunyamwera; Mengo; and Semliki Forest. In these tests over 100 MLD_{50} of each virus was present. The results suggested that the two strains might be strains of Ilhéus virus. In the case of the 6851 virus, Ilhéus immune serum protected all of the mice, whereas none of the other sera in the test afforded any protection. The test with the 5800 virus was not so clear-cut, but the delay in incubation period and time of death of the mice receiving virus plus Ilhéus immune serum was suggestive of protective effect.

Working from this lead, rabbits, which were found to be susceptible to Ilhéus virus by Koprowski and Hughes (1946), were inoculated intraperitoneally with infected mouse brain suspensions of the original Brazilian Ilhéus strain and the 5800 and 6851 strains in order to produce specific antisera to each. Sufficient preinoculation serum was collected from each rabbit to serve as a control in the tests employing the corresponding immune serum. The rabbits were exsanguinated between one and two months after inoculation. All sera were stored in sterile lusteroid tubes at $-20°C$.

The neutralizing capacity of each antiserum for each of the viruses was studied. In these tests tenfold dilutions of virus were mixed with an equal volume of undiluted rabbit immune serum. A control titration, employing the pre-immunization serum from the same rabbit, was made simultaneously. The sera were not inactivated. The serum-virus mixtures were incubated at 37°C. for an hour, after which they were kept in an ice bath during the time of inoculation. The serum-virus mixtures were inoculated intracerebrally into groups of six young adult mice. Titers were calculated by the method of Reed and Muench (1938). The neutralizing capacity was calculated as the difference between the titer of the virus in the pre-immunization sera and the post-immunization sera, and is recorded as the logarithm of the number of 50 per cent end point doses (MLD 50's) neutralized. Results of these tests are summarized in Table 2. The data show that each of the antisera neutralized significant amounts of each virus, and, taken as a whole, indicate that the agents were sufficiently similar to be considered as strains of the same virus.

Antigens were prepared to the Brazilian Ilhéus virus and to strains 5800 and

TABLE 2

Neutralizing capacity of rabbit immune sera prepared against three strains of Ilhéus virus and tested against the homologous and heterologous Ilhéus virus strains

(Neutralizing capacity is expressed as the logarithm of MLD_{50}'s neutralized)

Test virus	Animals immunized with Brazilian Ilhéus				Animals immunized with strain 5800		Animals immunized with strain 6851	
	Rabbit 7266	Rabbit 7267	Rabbit 7268	Rabbit 7269	Rabbit 7374	Rabbit 7375	Rabbit 7691	Rabbit 8070
Brazilian Ilhéus	>1.9*	>3.5	>1.9	>3.1	>1.9	>2.6	>3.6	>3.0
Strain 5800	1.8	3.1	1.6	1.6	3.1	2.2	2.6	>3.3
Strain 6851	>4.3	>3.9	2.7	4.1	>2.8	1.6	>4.3	>2.5

* In the instances where titrations were not carried past the end point, minimal figures for the quantity of virus neutralized are given.

6851, using baby mouse brain as starting material, according to the technique of Casals and Brown (1954). When tested for their ability to agglutinate baby chick red blood cells, all three strains showed optimum reactions at pH 6.8 and 4°C. The Brazilian Ilhéus and 6851 strains agglutinated beyond an antigen dilution of 1:3,200, while two different batches of strain 5800 had end points at 1:200 and 1:800 respectively. While such variations may be caused by inherent differences in strains, it is also possible that the time of harvesting the mouse brains may play a considerable part in determining the potency of antigen, and timing of the harvesting is difficult to standardize.

SUMMARY

Two strains of virus, isolated in 1954 from forest mosquitoes in Trinidad, B.W.I., have been shown by reciprocal cross-neutralization tests to be strains of Ilhéus virus. Both strains produce a hemagglutinating antigen, similar in reactivity to the antigen produced from the known Ilhéus strain. These isolations represent the second and third times the virus has been found in nature.

ADDENDUM

Since submitting this paper for publication, four more Ilhéus virus isolations from Trinidad mosquitoes have been made by the laboratory. All isolations were made in two-day-old mice inoculated with mosquitoes caught in the Rio Grande Forest about eight miles northeast of Sangre Grande. Strain one (TRVL specimen No. 10507) came from a mixed pool of 105 mosquitoes (21 species in nine genera) collected 16/XII/55; strain two (TRVL specimen No. 10554) came from a mixed pool of 233 mosquitoes (17 species in eight genera) collected 23/XII/55; strain three (TRVL specimen No. 10584) came from a mixed pool of 103 mosquitoes (13 species in seven genera) collected 3/1/56; and the fourth strain (TRVL specimen No. 10654) was isolated from a pool of seven *Psorophora ferox* collected 10/1/56. All of the mixed pools contained reasonably large numbers of *Aedes* and *Psorophora* species except No. 10584 which had 30 *Aedes* (*serratus*) and only one *Psorophora* (*lutzi*).

THE AUTHORS

REFERENCES

CASALS, J., AND BROWN, L. V., 1954. Hemagglutination with arthropod-borne viruses, *J. Exp. Med.* **99**: 429–449.

DOWNS, W. G., ANDERSON, C. R., AND THEILER, M., 1956. Neutralizing antibodies against certain viruses in the sera of residents of Trinidad, B.W.I., *Am. J. Trop. Med. & Hyg.*, **5**: 626–641.

KOPROWSKI, H., AND HUGHES, T. P., 1946. The virus of Ilhéus encephalitis. Physical properties, pathogenicity and cultivation, *J. Immunol.* **54**: 371–385.

LAEMMERT, H. W., JR., AND HUGHES, T. P., 1947. The virus of Ilhéus encephalitis. Isolation, serological specificity and transmission, *J. Immunol.* **55**: 61–67.

REED, L. J., AND MUENCH, H., 1938. A simple method of estimating 50 per cent endpoints, *Am. J. Hyg.* **27**: 493–497.

NEUTRALIZING ANTIBODIES AGAINST CERTAIN VIRUSES IN THE SERA OF RESIDENTS OF TRINIDAD, B.W.I.

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The Trinidad Regional Virus Laboratory is one of a series of field laboratories established by The Rockefeller Foundation to participate in a world-wide study of arthropod-borne virus diseases of man and animals. The other laboratories have been established in Poona, India, and Belem, Brazil, and, in addition, personnel have been assigned to already existing laboratories in Cairo, Egypt (U. S. Naval Medical Research Unit #3), and Johannesburg, South Africa (South African Institute for Medical Research). Trinidad was selected as a site for a field laboratory in part because of its convenient location at the hub of air and sea travel lanes of the West Indies and northern South America, and in part because the island has an equatorial climate with rain forest, savannah lands, and coastal marshes, and thus offers a variety of ecological zones. A good road network makes these various regions easily accessible. Furthermore, there is a reliable supply of solid CO_2 (dry ice) in Port-of-Spain.

Work at the laboratory in Trinidad was begun in early 1953, and by mid-1953 a serum-collecting program on the island was in progress. The present paper presents the results of neutralization tests on serum specimens collected in 1953 and early 1954. All neutralization tests were performed by The Rockefeller Foundation Virus Laboratories in New York.

TECHNIQUES OF THE NEUTRALIZATION TEST

The methods employed for the neutralization test were the same as those used in similar studies in other parts of the world (Smithburn *et al.*, 1954). In brief, groups of mice were inoculated with a mixture containing equal parts of the serum to be tested and a dilution of virus estimated to contain 100 LD_{50}. No test was considered valid unless the virus dose in a parallel titration was shown to be more than 50 LD_{50} per mouse. Results were interpreted according to the method used in similar previous studies.

SURVEY

Serum specimens were collected with the intention of sampling population groups from several different regions characteristic of the island. For this purpose the island was divided into ecological zones following a plan that was evolved during earlier malaria investigations on the island (Downs, Gillette and Shannon, 1943). In analyzing the incidence of malaria at that time, the island was divided into four zones: (a) a high rainfall-forest-cocoa zone; (b) an intermediate savannah zone; (c) a coastal swamp zone; and (d) urban zones. This plan was based on the belief that the distribution of malaria reflected the complex ecological situation on the island, and indeed it proved possible to show a rela-

TABLE 1

Results of neutralization tests performed on sera of Trinidad residents

Virus agent	Number of sera protective (numerator): Number tested (denominator)	% of sera showing protection
Yellow fever	111/875	12.7
Ilhéus	176/987	17.8
Dengue I (Hawaiian)	272/1034	26.3
Dengue II (New Guinea)	68/245	27.8
Venezuelan equine encephalitis	11/160	6.9
St. Louis encephalitis	15/348	4.3
Uganda S	3/100	3.0
West Nile	6/91	6.6
Semliki Forest	1/159	0.7
Ntaya	4/24	16.6
Japanese B encephalitis	1/98	1.0
Eastern equine encephalitis	0/124	0.0
Bwamba	0/50	0.0
Zika	0/15	0.0
Anopheles A	0/49	0.0
Western equine encephalitis	0/6	0.0
Bunyamwera	0/190	0.0

tionship between malaria incidence, on the one hand, and vector biology, topography, rainfall, and flora, on the other.[1]

It was felt that this plan, or a similar type of zoning, might help in orienting future virus research, even though the epidemiology of arthropod-borne virus diseases offers many possible points of difference from that of malaria because of its dependence on various insect vectors, the presence of intermediate hosts among animals or birds, and differing basic cycles of transmission. Accordingly, in the present survey care was taken to obtain serum specimens from the four zones mentioned above, in order to explore all the possibilities offered by the island.

A total of 1,055 serum specimens are considered in the present analysis. Table 1 presents the results of 4,555 neutralization tests performed on them. Table 2 is a preliminary attempt to interpret the general picture of virus activity in Trinidad. The results presented in Tables 1 and 2 require further comment and analysis.

Smithburn (1954) and Smithburn, Kerr and Gatne (1954) discuss the significance of cross-reactions and multiple positives in neutralization tests. Cross-reactions may make the reactions, as determined in an individual serum specimen, difficult to interpret, and such is indeed the case with the St. Louis, West

[1] Pittendrigh (1950a, 1950b) has published detailed discussions of the ecological divergences of two bromeliad breeding anophelines in Trinidad which serve as an excellent introduction to some aspects of the local biological associations. In his consideration of bromeliads and mosquitoes (*Kerteszias*) (1948), he divides the island into seven climatic zones.

TABLE 2

Occurrence in Trinidad, B.W.I. of virus agents as deduced from results of neutralization tests

Virus	Status in Trinidad, B.W.I.
Yellow fever	Present, widespread.
Ilhéus	Present, presumably widespread and common.
Dengue (Strains 1 and II)	Present, presumably widespread and common.
Venezuelan equine encephalomyelitis	Tests indicate presence some years ago, with no evidence of recent activity.
St. Louis encephalitis	Evidence to date indicates probable presence although there exists the possibility of cross-immune reactions confusing interpretations.
Uganda S, West Nile, Semliki, Ntaya, Japanese B	Inconclusive evidence for presence, complicated by possibility of cross-immune reactions.
Eastern equine encephalitis	Evidence to date indicates probable presence.
Bwamba, Zika, Anopheles A, Western equine encephalitis, Bunyamwera	Evidence to date indicates probable absence.

Nile, Ntaya, and Japanese B encephalitis reactives described in greater detail below.

In the Trinidad analyses, high immunity rates are encountered for yellow fever, dengue and Ilhéus viruses and numerous serum specimens show clear-cut immunity to more than one of these three (Table 3). Under such circumstances, can the results of testing be interpreted rigidly enough to permit an analysis of distribution of these agents? We feel that an analysis can be carried out safely for several reasons. First, all three of these agents have been isolated in Trinidad by this laboratory (yellow fever: Anderson, Spence, and Downs, 1955; dengue: Anderson, Downs, and Hill, paper in preparation; Ilhéus: Anderson, Aitken, and Downs, 1956). Secondly, there are numerous sera that neutralize only a single virus (Table 3). Thirdly, many of the multiple reactions are in individuals from areas of the island where immunity rates to all three viruses are higher than the over-all rates from which Table 3 is constructed. It is probable that many of the multiple reactions from such areas simply mean infection with two or even three of the viruses during the course of the individual's lifetime. While the possible presence of cross-immune reactions is not denied, it is considered improbable that such cross-immune reactions have seriously affected the gross interpretation of results of tests involving yellow fever, dengue and Ilhéus viruses, inter se, in these analyses.

Table 3 presents data on multiple reactions to yellow fever, dengue, and Ilhéus viruses.

TABLE 3

Multiple reactions encountered in the sera of 815 Trinidad residents which were exposed in neutralization tests to three agents: yellow fever, dengue I, and Ilhéus

	Number	Per cent
Yellow fever:		
Negative*	704	86.4
Positive for yellow fever only	38	4.7
Positive for YF and dengue	27	3.3
Positive for YF and Ilhéus	12	1.5
Positive for YF, dengue and Ilhéus	34	4.2
	815	100.1
Dengue I:		
Negative*	530	65.0
Positive for dengue only	186	22.8
Positive for dengue and YF	27	3.3
Positive for dengue and Ilhéus	38	4.7
Positive for dengue, Ilhéus and YF	34	4.2
	815	100.0
Ilhéus:		
Negative*	655	80.3
Positive for Ilhéus only	76	9.3
Positive for Ilhéus and YF	12	1.5
Positive for Ilhéus and dengue	38	4.7
Positive for Ilhéus, YF and dengue	34	4.2
	815	100.0

* Inconclusives (reactions showing partial protection) are included in above negatives.

DISCUSSIONS OF INDIVIDUAL DISEASES

Yellow fever. Although this disease was last reported in Trinidad in 1914, neutralization tests performed on sera collected in 1953 showed, in addition to high percentages of immunes in older people from rural areas, eight immunes among young people between 15 and 20 years of age from forested regions of east-central Trinidad. Since no immunes were found in this area in children under 15 years of age, no evidence is at hand to show that yellow fever has been active during the 15 years preceding 1954. Immunity rates in urban Port-of-Spain were much lower. No immunes were encountered in 172 individuals under 40 years of age and only four were found among nine individuals over 40 years of age. In 1954, an outbreak of yellow fever in Trinidad was actually followed by this laboratory (Downs, Aitken, and Anderson, 1955).

One interesting feature of the sex distribution of yellow fever immunity in rural Trinidad, shown in Table 4, is the close correspondence of immunity rates in males and females. It has been found in South American jungle yellow fever

TABLE 4

Yellow fever: Analysis by age and sex of protection tests performed on 694 human serum specimens from rural Trinidad

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.	Total in group	% pos.	Pos.	Neg.	Total in group	% pos.		
1–4	0	5	5	0	0	5	5	0	10	0
5–9	0	26	26	0	0	24	24	0	50	0
10–14	0	40	40	0	0	34	34	0	74	0
15–19	3	21	24	12.5	5	71	76	7.1	100	8.0
20–29	1	61	62	1.6	5	82	87	5.7	149	4.2
30–39	6	47	53	11.3	9	54	63	14.3	116	12.9
40–49	11	30	41	26.8	11	30	41	26.8	82	26.8
50 and up	35	33	68	51.5	21	24	45	46.5	113	49.6
	56	263	319	17.6	51	324	375	13.6	694	15.4

that males are much more affected, presumably because of increased exposure attributable to occupation. Two possible explanations may be offered for the lack of this distinction in Trinidad. One is that *Haemagogus* mosquitoes in Trinidad bite freely at ground level in the forests and at roadsides and in clearings (Downs *et al.*, 1955), and, since rural Trinidadians often live in or close to forests, both sexes may therefore be more or less equally exposed to *Haemagogus*. The other possibility is that, owing to the wide distribution of *Aedes aegypti* throughout the rural areas, including a high proportion of houses deep in the forest, the infections brought in by the working man from the forest may be secondarily established in the home, again equalizing exposure of the sexes.

Almost the entire population of Trinidad has now been immunized against yellow fever, in an island-wide program begun in July, 1954 and still in progress, so that further serological studies of yellow fever distribution and incidence in the population will not be practicable.

Ilhéus. This virus was isolated from forest mosquitoes (Laemmert and Hughes, 1947) during the course of a jungle yellow fever study program near Ilhéus, Bahia, Brazil. The virus possesses serological specificity and produces encephalitis in laboratory animals. Neutralizing antibody was found in human beings from the Ilhéus region. No cases in human beings under natural conditions have ever been seen, although induced disease in human beings has been described (Southam and Moore, 1951). Ilhéus virus has been isolated twice from mosquitoes in Trinidad during 1954, captures being made in the lowland-heavy rainfall-forested areas (Anderson, Aitken, and Downs, 1956).

In the lowland, heavy rainfall, forested areas of Trinidad, infection with Ilhéus virus appears to be a frequent occurrence, since immunity rates are high. Rates are not nearly so high in the forested areas with heavy rainfall in the mountains of the northern range, and are almost nil in cities. Tables 5, 6, and 7 present the Ilhéus immunity pattern in several different groupings of people.

TABLE 5

Ilhéus virus: analysis by age and sex of protection tests performed on 291 human serum specimens collected from the Biche region (lowland forest—heavy rainfall—cocoa area)

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.*	Total in group	% pos.	Pos.	Neg.*	Total in group	% pos.		
1–4	0	5	5	0	0	5	5	0	10	0
5–9	1	24	25	4.0	0	24	24	0	49	2.0
10–14	4	22	26	15.4	0	27	27	0	53	7.5
15–19	3	5	8	37.5	8	8	16	50.0	24	45.6
20–29	5	5	10	50.0	4	17	21	19.1	31	29.1
30–39	15	5	20	75.0	9	17	26	34.6	46	52.1
40–49	7	3	10	70.0	4	9	13	30.8	23	47.7
50 and up	22	11	33	66.6	7	15	22	31.8	55	52.6
	57	80	137	41.7	32	122	154	24.1	291	30.6

* 16 inconclusive tests to Ilhéus are included in above negatives.

TABLE 6

Ilhéus virus: analysis by age and sex of protection tests performed on 89 human serum specimens collected from the Arima valley (montane forest—heavy rainfall—cocoa area)

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.*	Total in group	% pos.	Pos.	Neg.*	Total in group	% pos.		
1–4	0	0	0	—	0	0	0	—	0	—
5–9	0	1	1	—	0	0	0	—	1	—
10–14	0	12	12	—	1	1	2	—	14	7.1
15–19	0	5	5	—	0	4	4	—	9	—
20–29	2	9	11	18.2	0	6	6	—	17	11.8
30–39	0	7	7	—	1	2	3	—	10	10.0
40–49	5	10	15	33.3	1	3	4	—	19	31.6
50 and up	3	11	14	21.4	0	5	5	—	19	15.8
	10	55	65	15.4	3	21	24	14.3	89	14.6

* 4 inconclusive tests to Ilhéus are included in above negatives.

The differences in immunity rates between lowland forest areas and urban areas are striking. Furthermore, rates are highest in males, with a sharp rise after the age of 15. The fact that the young men begin to work actively and steadily in the forest at about this age suggests that Ilhéus virus infection is forest acquired.

The differences between lowland and montane heavy-rainfall areas, brought out in Tables 5 and 6, have been further confirmed by tests run on specimens from Cumaca, another montane region. Of 166 Cumaca specimens analyzed, only 11, or 6.6 per cent, neutralized Ilhéus virus.

TABLE 7

Ilhéus virus: analysis by age and sex of protection tests performed on 115 human serum specimens collected from Port-of-Spain residents

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.	Total in group	% pos.	Pos.	Neg.	Total in group	% pos.		
1–4	0	0	0	—	0	0	0	—	0	—
5–9	0	0	0	—	0	0	0	—	0	—
10–14	0	0	0	—	0	0	0	—	0	—
15–19	0	14	14	0	0	22	22	0	36	0
20–29	0	31	31	0	0	20	20	0	52	0
30–39	0	6	6	—	0	12	12	0	18	0
40–49	0	2	2	—	0	3	3	—	5	—
50 and up	0	3	3	—	0	1	1	—	4	—
	0	56	56	0	0	58	58	0	115	0

The reason for this difference in rates of immunity to Ilhéus between lowland and montane forested areas is not yet apparent, but speculation is possible. Laemmert and Hughes (1947) isolated Ilhéus virus from the serum of a monkey inoculated earlier with mixed pools of *Aedes* and *Psorophora* mosquitoes. Although several species of each genus were included, *Aedes serratus* and *Psorophora ferox* predominated. Both of these species breed in ground water, and the same is true of the other *Psorophora* species in the pool. About the *Aedes* we cannot say, but several species which breed in ground water do exist in the Ilhéus region. All of the above-mentioned species are common in the lowland forest of Trinidad, but exist in smaller numbers in the mountainous regions where drainage is better. The low rates of immunity in the latter areas may therefore be due to the scarcity or even absence of a suitable vector.

Dengue. This disease has hardly been recognized in Trinidad. Many physicians say the disease does not occur; others affirm that it does, and call attention to small outbreaks which they have observed (often in recently arrived Europeans) and which appear to them to be dengue outbreaks. No Health Department records of outbreaks, large or small, can be found. Neutralization tests performed indicate high rates of immunity in both rural and urban areas. Here again it should be noted that *Aedes aegypti* is common not only in urban but also in rural areas. Over 70 per cent of the premises in some rural areas are infested. This situation is changing rapidly, however, now that an *Aedes aegypti* eradication program for the island has been started. Tables 8 and 9 present dengue immune rates (dengue I) from rural and urban zones. Males and females are about equally affected, and the immunity rates are actually higher in the rural area of Biche than in Port-of-Spain. Table 1 shows further that both dengue I and dengue II viruses are neutralized by a high proportion of sera from Trinidad.

The dengue immune rates shown in Tables 8 and 9 are certainly minimal rates. Neutralization tests were performed with dengue I and dengue II virus

TABLE 8

Dengue virus (strain I): analysis by age and sex of protection tests performed on 289 human serum specimens collected from the Biche region (rural)

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.*	Total in group	% pos.	Pos.	Neg.*	Total in group	% pos.		
1–4	0	5	5	0	0	5	5	0	10	0
5–9	1	24	25	4.0	0	24	24	0	49	2.0
10–14	5	21	26	19.3	10	17	27	36.9	53	28.2
15–19	3	5	8	37.5	9	7	16	56.2	24	50.0
20–29	2	8	10	20.0	13	8	21	61.8	31	48.3
30–39	14	6	20	70.0	14	12	26	53.5	46	60.9
40–49	8	2	10	80.0	9	4	13	69.5	23	73.5
50 and up	27	6	33	81.6	18	2	20	90.0	53	85.0
	60	77	137	43.8	73	79	152	48.0	289	45.8

* 33 "inconclusives" are included in negatives.

TABLE 9

Dengue virus (strain I): analysis by age and sex of protection tests performed on 165 human serum specimens collected from Port-of-Spain (Urban)

Age group	Male				Female				Grand total	% pos.
	Pos.	Neg.*	Total in group	% pos.	Pos.	Neg.*	Total in group	% pos.		
1–4	0	0	0	—	0	0	0	—	0	—
5–9	0	36	36	0	0	14	14	0	50	0
10–14	0	0	0	—	0	0	0	—	0	—
15–19	2	12	14	14.3	1	21	22	4.5	36	8.3
20–29	8	24	32	25.0	7	13	20	35.0	52	28.8
30–39	1	5	6	16.7	5	7	12	41.5	18	33.0
40–49	0	3	3	—	0	2	2	—	5	—
50 and up	1	2	3	33.3	0	1	1	—	4	25.0
	12	82	94	12.7	13	58	71	18.3	165	15.2

* 19 "inconclusives" are included in negatives.

strains, neither of which can be considered as "native," and many tests reported as inconclusive or even negative actually show considerably prolonged average survival times of inoculated mice.

A strain of dengue (strain TRVL #1751) was isolated in Trinidad from a human patient in 1953 (Anderson, Downs, and Hill, in preparation). It has not yet been fully typed in relationship to known dengue I and dengue II but has already been successfully adapted to adult mice by the intracerebral route. It is proposed to study the local dengue situation further, using the local dengue strain as the test virus.

Venezuelan equine encephalitis. This disease was last known to be in Trinidad in 1943–44 (Gilyard, 1944, 1945), when an epidemic occurred in which two persons died. Blood specimens were collected from 58 presumably healthy individuals in the Penal-Siparia district in 1943, and 12 of these (21 per cent) had immune bodies to VEE in their serum (Pewan, unpublished data). Sanmartin *et al.* (1954) show that epidemics of this disease among human beings may sometimes be mistaken for influenza or dengue, and that subsequently many persons immune to VEE can be demonstrated.

In the present analysis, 160 neutralization tests were performed, largely on specimens from people of the San Rafael and Arima Valley regions. There were 11 positives, all in individuals over 15 years of age. Thirty-six tests were also run on equines. Two were positive, both in old donkeys already alive in 1943. Thus, these present testings do not indicate either any later occurrence or any continued activity of the disease after 1944.

St. Louis encephalitis. In 348 neutralization tests, almost all of them performed on specimens from heavy-rainfall, forested areas, only 15 positive reactors were encountered. Among these the frequency of cross-immune reactions (sera showing protective antibodies against one or more virus agents) was high (Table 10). However, although no consistent pattern was demonstrated, three of the sera (D.A., N.C., and H.A.) failed to show protective antibodies against any of the other viruses tested. It is perhaps significant that, with only 15 positive results in 384 sera tested, 11 were found in 32 sera from the San Rafael region. It would appear possible that St. Louis virus infections have occurred in Trinidad.

A labile factor in serum, enhancing the protective effect of antibody, has

TABLE 10

Serological reactions exhibited by serum specimens from Trinidad residents which neutralize St. Louis virus

Name	Age	Sex	Locality	Y.F.	Sem.	Bun.	Ilh.	EEE	WEE	VEE	Dengue I
AC	49	F	San Rafael	Neg.	Neg.	Neg.	Neg.	—	—	Neg.	Pos.
TP	40	F	San Rafael	Pos.	—	—	Pos.	Neg.	—	—	Inc.
DA	45	F	San Rafael	Neg.	—	—	Neg.	Neg.	—	—	Neg.
LP	46	M	San Rafael	Neg.	—	—	Pos.	Neg.	—	—	Neg.
KA	16	F	San Rafael	Neg.	Neg.	Neg.	Pos.	—	—	Neg.	Neg.
RR	84	M	San Rafael	Neg.	Neg.	Neg.	Neg.	—	—	Neg.	Inc.
JM	24	M	San Rafael	Neg.	—	—	Pos.	Neg.	—	Neg.	Neg.
CG	70	M	San Rafael	Neg.	Neg.	Neg.	Pos.	—	—	Neg.	Inc.
MR	38	F	San Rafael	Neg.	—	—	Neg.	Neg.	Neg.	Pos.	Neg.
VM	47	F	San Rafael	Pos.	Neg.	Neg.	Neg.	—	—	Neg.	Neg.
CD	20	M	Valencia	Neg.	—	—	Pos.	—	—	Pos.	Neg.
JP	55	M	Cumaca	Pos.	—	—	Neg.	—	—	—	Inc.
NC	51	M	Cumaca	Neg.	—	—	Neg.	—	—	—	Neg.
HA	32	F	Cumaca	Neg.	—	—	Neg.	—	—	—	Neg.
NH	45	M	Cumaca	Pos.	—	—	Pos.	—	—	—	Pos.
				4/15	0/5	0/5	7/15	0/5	0/1	2/8	2/15

TABLE 11

Serological reactions exhibited by serum specimens from Trinidad residents which neutralize West Nile virus

Name	Age	Sex	Locality	Y.F.	Bw.	Zi.	Bun.	Ug.S	Ilh.	St.L.	D.I	D.II	Jap. B
EB	40	F	San Rafael	Neg.	—	—	—	—	Pos.	—	Pos.	Pos.	Pos.
EC	30	F	Sangre Grande	—	—	—	—	—	Pos.	Neg.	Pos.	Inc.	Neg.
MM	60	M	Biche	Pos.	Neg.	Neg.	Neg.	Neg.	Pos.	—	Pos.	Pos.	Neg.
DL	35	M	Biche	Pos.	—	—	—	—	Pos.	Inc.	Pos.	—	Neg.
RS	36	M	Biche	Inc.	—	—	—	—	Pos.	—	Pos.	—	Neg.
EJ	22	M	Biche	Neg.	—	—	—	—	Pos.	Neg.	Neg.	—	—

been postulated. The above tests were performed on sera several months old without addition of a labile factor. Since under these circumstances the validity of a negative reaction can be questioned, St. Louis immune rates may actually be higher.

Uganda S. Of the three specimens positive for Uganda S among 100 tested, one, from a 19-year-old female, was positive also for yellow fever, Ilhéus and dengue I; one, from a 67-year-old male, was positive also for yellow fever, Ilhéus, dengue I and dengue II; and one, from a 50-year-old female, was positive also for yellow fever and dengue I and inconclusive (showing partial protection) to Ntaya and dengue II. To draw conclusions from these data about the presence or absence of Uganda S would be unwarranted, because MacNamara (1953) has described cross neutralization of Uganda S virus by yellow fever immune serum.

West Nile. Table 11 illustrates the frequency of cross-reactions occurring among the six Wext Nile positive serum specimens. Protection against Ilhéus virus was present in all of these positives and against dengue I in five of the six. Interpretation of these West Nile immunes is not attempted.

Semliki Forest. The one Semliki positive serum reacted also to yellow fever, Ilhéus, and dengue I. A specimen of donkey serum from Guayaguayare also showed partial protection against Semliki virus. Further interpretation of these results is not attempted at present.

Ntaya. There is the following accessory data on the four positives among 24 sera tested against Ntaya virus: one, from a 68-year-old female, was positive also for yellow fever, dengue I, and dengue II and partially protective against Zika and Uganda S; the second, from a 39-year-old male, was positive also for Ilhéus and dengue II and partially protective against dengue I; the third, from a 34-year-old female, was protective only against dengue I; and the fourth, from a female of unstated age, was protective only against dengue I and dengue II. Whether the above tests give actual evidence of the presence of Ntaya virus cannot be definitely stated at this time because of the possibility of cross-immune reactions, particularly with dengue.

Japanese B encephalitis. The one Japanese B positive reactor among 98 sera

tested was derived from a 40-year-old female and was positive also for West Nile, Ilhéus, dengue I, and dengue II. The significance of this lone positive may be questioned.

Eastern equine encephalitis. No positive reactions for Eastern equine encephalitis were encountered among 124 human specimens tested. However, of 22 equine sera tested, one, from a donkey in Cumaca, gave a positive reaction. This finding was repeated and confirmed on another blood specimen taken from the same animal at a later date.

Other viruses. No positives were encountered to Bwamba (0/50), Zike (0/15), Anopheles A (0/49) and Western equine encephalitis (0/6), although in some instances the total number of specimens was so small that further exploratory testing is indicated.

DISCUSSION OF ECOLOGICAL ZONES

In interpreting the distribution of certain diseases within a given area, it is sometimes helpful to divide the area into zones based upon physico-geographical and climatological features. Indeed, when the diseases in question are spread by insect vectors and have lower animal reservoirs, such a division is, in effect, an ecological zoning which is tailored to fit vector and host and into which distribution of disease fits more or less perfectly.

The construction of ecological zoning involves the danger of over-simplification, however. There are three sets of data to be considered—those bearing upon the physico-geographical and climatological features of the area; those relating to vector (or suspect vector) and host (or suspect host) distribution; and those

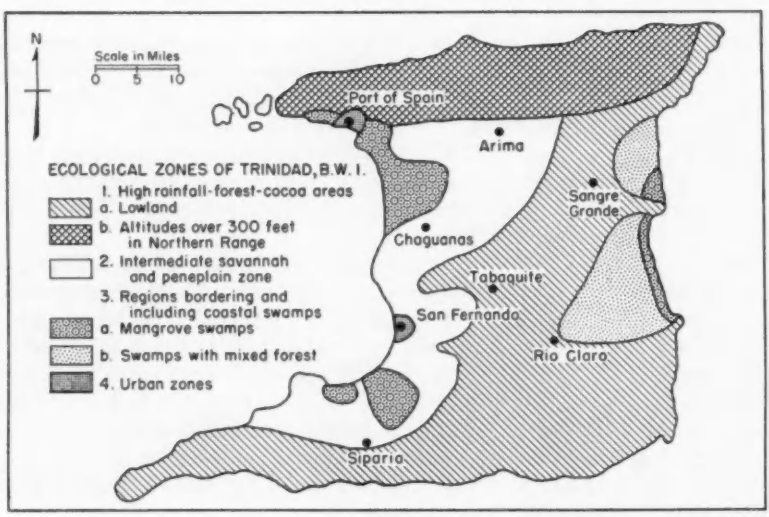

FIG. 1

concerning disease distribution—and the automatic tendency is to try to harmonize them. This may result in small areas of differing ecological relationships being submerged in the large area of a more general zone. An intimate knowledge of the geography and general ecology of the zones under consideration will sometimes help to explain apparent discrepancies. In Trinidad, the effects of considerable rainfall differences from the east to the west of the island (see Figure 2) must be kept constantly in mind in ecological zoning. Also important is the effect of elevations up to 3,000 feet in the northern range of mountains, with accompanying rainfall, temperature, and humidity changes, and with the better drainage of hilly country.

For the purpose of characterizing and describing our data on protection tests with yellow fever, Ilhéus, and dengue, a provisional ecological zoning of Trinidad is presented in Figure 1. This zoning is based mainly upon physico-geographical and climatological features and epidemiological findings, and is more detailed than the one, mentioned above, which was derived from malaria investigations. Vector and host distribution have not been considered fully, because our knowl-

TABLE 12

Results of protection tests performed on sera of Trinidad residents against yellow fever, Ilhéus and dengue I viruses, arranged in ecological zones

Ecological zone and locality	Number of specimens studied	Number protective (numerator) and Number tested (denominator)		
		Yellow fever	Ilhéus	Dengue I
1. *High rainfall—forest—cocoa areas*				
(a) Lowland				
Sangre Grande	47	5/44	12/47	8/47
San Rafael	144	22/144	44/144	27/144
Biche	291	63/286	89/291	133/289
Sub-totals	482	90/474	145/482	168/480
(b) Montane				
Arima Valley	90	9/89	13/89	8/88
Cumaca	166	—	11/166	19/166
Sub-totals	256	9/89	24/255	27/254
2. *Intermediate savannah and peneplain zone:*				
Arima	66	2/64	2/66	28/65
3. *Regions bordering on and including coastal swamps*				
(a) Mangrove				
Chaguanas	70	6/67	5/70	24/70
(b) Swamps with mixed forest				
No data	—	—	—	—
4. *Urban:* Port-of-Spain	181	4/181	0/114	25/165
Totals	1,055	111/875	176/987	272/1034

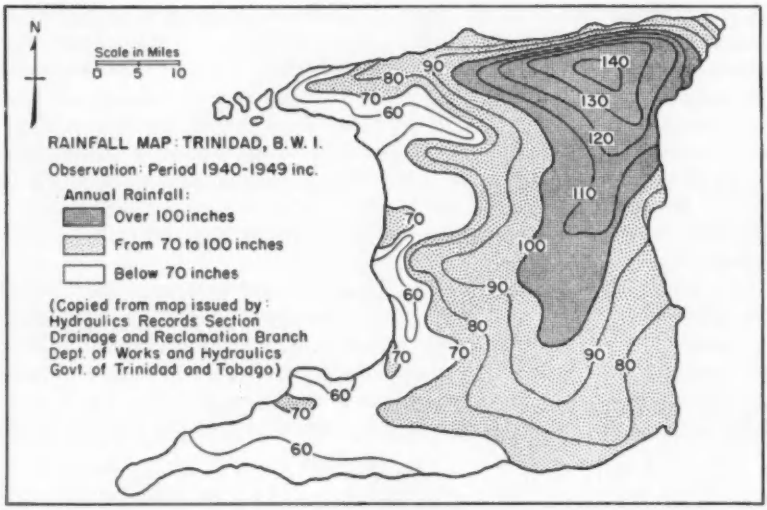

FIG. 2

edge of vectors and hosts is incomplete. Table 12 presents the epidemiological data arranged according to the ecological zoning of Figure 1.

The following brief notes summarize and attempt to explain the interrelationships of zones and diseases. It should be noted here that the human population of Trinidad is by no means static and that proper residence histories are not always easy to obtain. In some instances, therefore, infections supposedly acquired in one area may actually have been acquired elsewhere, with the result that a given area and the disease contained therein may in reality be less sharply delimited than is suggested here.

Zone 1. High rainfall-forest-cocoa areas.

a. *Lowland.*
Ilhéus: rates high—Drainage is often poor and certain ground breeding forest mosquitoes are abundant. Also many inhabitants work in forests.
Dengue: rates high—*Aedes aegypti* is common in houses in these regions.
Yellow fever: rates high—Monkeys and *Haemagogus* are present in the forest, and *Aedes aegypti* in the houses, and many inhabitants work in forests.

b. *At altitudes of over 300 feet in Northern Range.*
Ilhéus: rates are much lower—Drainage is better and ground breeding forest mosquitoes are not favored. Many inhabitants in this region also work in forests.
Dengue: rates are low—*Aedes aegypti* is uncommon or absent in houses in much of this region.
Yellow fever: rates are average—Monkeys and *Haemagogus* exist in the

forests. Beebe (1952) has published a detailed general ecological study of the middle zone of the Arima Valley, a typical "1b" locality.

Zone 2. Intermediate savannah and peneplain zone.

Ilhéus: rates are low—There is absence of forest and absence of conditions proper for ground breeding forest mosquitoes.
Dengue: rates are high—*Aedes aegypti* is common in houses.
Yellow fever: rates are average, with *Aedes aegypti* common in houses.

Zone 3. Regions bordering on and including coastal swamps.

a. *Mangrove swamp.*
Ilhéus: rates are low—There is absence of conditions favorable for ground breeding forest mosquitoes.
Dengue: rates are high—*Aedes aegypti* is common in houses.
Yellow fever: rates are average—*Aedes aegypti* is common in houses.

b. *Swamps with mixed forest.*
No data. These forests are almost unpopulated and are visited only by forest workers and huntsmen. The forests are largely in the Nariva Swamp and the Manzanilla Windbelt reserve. One would expect Ilhéus and jungle yellow fever to be present and dengue absent.

Zone 4. Urban zones.

Ilhéus: rates nil—There is absence of conditions favorable for ground breeding forest mosquitoes.
Dengue: rates are moderate—*Aedes aegypti* is common in houses.
Yellow fever: rates are low—*Aedes aegypti* is common in houses, but urban yellow fever outbreaks are not common presumably.

The above provisional scheme fits Ilhéus data well. Dengue carries across most boundaries with its nearly ubiquitous (as far as human habitations are concerned) vector, *Aedes aegypti*. In the one zone (1b) where *Aedes aegypti* is less frequently found, dengue rates are appreciably lower. Yellow fever is common in all areas except urban.

Venezuelan equine encephalitis studies may throw additional light on an ecological zoning, but data on VEE neutralization are still too fragmentary to be of use. Similarly, the distribution of St. Louis immunity may be of interest when more complete data are at hand.

SUMMARY

Blood samples taken from 1,055 indigenous residents of Trinidad, B.W.I., were tested for neutralizing antibody against 17 different viral agents known or believed to be arthropod-borne, in 4,555 protection tests. Yellow fever, Ilhéus and dengue are, or have been, on the basis of protection tests, presumably present, widespread, and common in Trinidad. A yellow fever outbreak occurred during the course of investigations in 1954. There is no evidence that Vene-

zuelan equine encephalitis has been present on the island since the last reported outbreak in 1943. A small number of sera were found which neutralized St. Louis encephalitis virus. No interpretation of this result is attempted. Although no human sera neutralized Eastern equine encephalitis virus, two different specimens of serum from one donkey neutralized the virus in two different tests. Interpretation of tests against Uganda S, West Nile, Semliki, Ntaya, and Japanese B is complicated by the possibility of interfering cross-immune reactions. All tests with Bwamba, Zika, Anopheles A, Western equine encephalitis, and Bunyamwera viruses were negative. A provisional ecological zoning of the island is presented, based upon distribution of immunity against several virus agents.

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REFERENCES

Anderson, C. R., Aitken, T. H. G., and Downs, W. G. The isolation of Ilhéus virus from wild caught forest mosquitoes in Trinidad, *Am. J. Trop. Med. Hyg.* **5:** 621–625.

Anderson, C. R., Spence, L., and Downs, W. G., 1954. Report of a case of yellow fever in Trinidad, B.W.I., *Carib. Med. J.* **16:** 68–70.

Beebe, W., 1952. Introduction to the ecology of the Arima Valley, Trinidad, British West Indies, *Zoologica*, **37:** 157–183, pl. 5.

Downs, W. G., Gillette, H. P. S., and Shannon, R. C., 1943. A malaria survey of Trinidad and Tobago, British West Indies, *J. Nat. Mal. Soc.*, supplement to Vol. 2: 1–44.

Downs, W. G., Aitken, T. H. G., and Anderson, C. R., 1955. Activities of the Trinidad Regional Virus Laboratory in 1953 and 1954 with special reference to the yellow fever outbreak in Trinidad, British West Indies, *Am. J. Trop. Med. & Hyg.* **4:** 837–843.

Gilyard, R. T., 1944. Mosquito transmission of Venezuelan virus equine encephalomyelitis in Trinidad, *Bull. U. S. Army Med. Dept.* **75:** 96–107.

Gilyard, R. T., 1945. A clinical study of Venezuelan virus equine encephalomyelitis in Trinidad, British West Indies, *J. Am. Vet. Assn.* **106:** 267–277.

Laemmert, H. W., Jr., and Hughes, T. P., 1947. The virus of Ilhéus encephalitis. Isolation, serological specificity and transmission, *J. Immunol.* **55:** 61–67.

MacNamara, F. N., 1953. Uganda S and yellow fever viruses. A slight relationship shown by experiments in rhesus monkeys and white mice, *Brit. J. Exp. Path.* **34:** 392–399.

Pittendrigh, C. S., 1948. The bromeliad-anopheles-malaria complex in Trinidad. I. The bromeliad flora, *Evolution* **2:** 58–89.

Pittendrigh, C. S., 1950a. The ecoclimatic divergence of *Anopheles bellator* and *Anopheles homunculus*, *Evolution* **4:** 43–63.

Pittendrigh, C. S., 1950b. The ecotopic specialization of *Anopheles homunculus*; and its relation to competition with *A. bellator*, *Evolution* **4:** 64–78.

Sanmartin, C., Barbier, C., Groot, H., and Osorno-Mesa, E., 1954. Human epidemic in

Colombia caused by the Venezuelan equine encephalomyelitis virus, *Am. J. Trop. Med. & Hyg.* **3:** 283–293.

SMITHBURN, K. C., 1954. Antigenic relationships among certain arthropod-borne viruses as revealed by neutralization tests, *J. Immunol.* **72:** 376–388.

SMITHBURN, K. C., KERR, J. A., AND GATNE, P. B., 1954. Neutralizing antibodies against certain viruses in the sera of residents of India, *J. Immunol.* **72:** 248–257.

SOUTHAM, C. M., AND MOORE, A. E., 1951. West Nile, Ilhéus and Bunyamwera virus infections in man, *Am. J. Trop. Med.* **31:** 724–741.

TRINIDAD AND TOBAGO, 1915. Report of the Surgeon-General on the Medical Department for the year 1913–14. Council Paper No. 205 of 1914, pp. 8–9, 12–18. Gov't Printing Office, Port-of-Spain, Trinidad, B.W.I. (Note: see reprinting in *Carib. Med. J.*, 1954, **16:** 60–86.)

STUDIES ON AN OUTBREAK OF INFLUENZA IN BEIRUT, LEBANON

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An outbreak of upper respiratory infection[1] appeared at the American University of Beirut during the winter of 1953–54,[2] and involved a large number of students and employees. It occurred in two distinct waves. The first wave started during the last week of November and reached its peak in the second week of December 1953, to decline thereafter. The second wave commenced during the second week of January 1954, showed a sharp rise and disappeared during the first week of February (Fig. 1). The fact that the number of patients who suffered from upper respiratory infections at this time of the year exceeded greatly the number involved during same period in the previous year, made it seem opportune to carry out clinical and serological studies. In this outbreak while all age groups and both sexes were equally involved, only a limited number, young adult males admitted to the Gulbenkian Infirmary for treatment, were studied.

CLINICAL PRESENTATION

In the majority of cases, onset of the illness was sudden with involvement of the upper respiratory tract, accompanied by constitutional symptoms.

1. *Symptoms referable to the upper respiratory tract.* These included nasal obstruction, rhinorrhea with mucoid and/or mucopurulent discharge. In most cases a mild or a severe congestion of the throat was observed which usually was accompanied by hypertrophy of lymph follicles. Palpable sub-mandibular or cervical nodes were occasionally found. Hoarseness was not a usual finding. Cough was commonly present and substernal soreness was frequently observed. Auscultation of the chest was negative except for occasional harsh breath sounds and wheezes.

2. *Constitutional symptoms.* These included general malaise, headache, muscular and joint pains, photophobia, conjunctival injection, chilliness, fever, sweating, dizziness, anorexia and, in rare instances, diarrhea and/or vomiting. No rash was present. In the majority of cases, both upper respiratory tract and constitutional symptoms were present with equal severity, while in others either one of these groups of symptoms predominated.

The general impression was that patients involved in the second wave of this outbreak presented a longer period of illness, and experienced mild complications (sinusitis, otitis media etc.,) more frequently, than those involved in the first wave. Aside from these, the clinical course presented by patients during each of the two waves was quite similar.

[1] The term "upper respiratory infection" is used in this paper to indicate a variety of conditions, i.e., influenza, common cold, and other respiratory infections of unknown etiology.

[2] According to the reports of local physicians (personal communication), this epidemic affected concomitantly other areas in Lebanon.

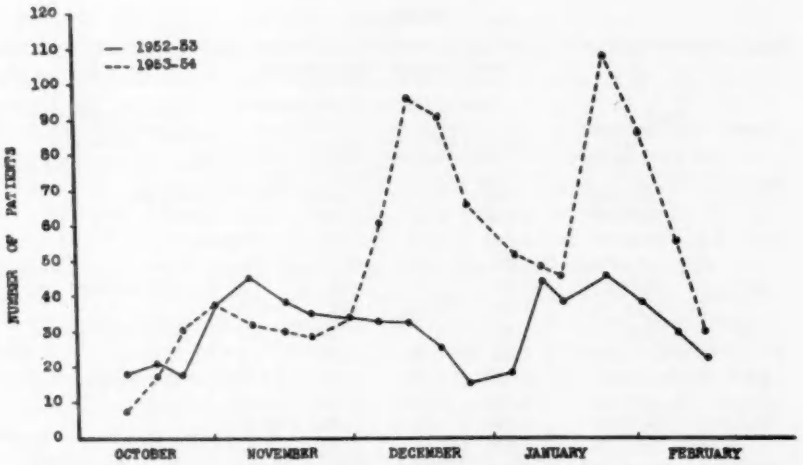

FIG. 1. The incidence of upper respiratory infection in patients that attended the Gulbenkian Infirmary during winter months of 1952–53 and 1953–54.

Pneumonia and/or other serious complications were remarkably absent. During the period of this epidemic two frank cases of pneumonia appeared, but in neither of the cases was there any indication that the disease developed as a complication to a previously acquired upper respiratory tract infection.

Leukocyte counts were carried out on most of the patients. The results showed either a normal count or leukopenia with relative lymphocytosis. Tests to determine erythrocyte sedimentation rates, and to measure the amount of cold agglutinins in serum samples, were also done on a few individuals. The results indicated normal blood sedimentation rates, and absence of increase of cold agglutinins in the blood samples obtained during and after the course of illness. The treatment in all of the cases was symptomatic and no antibiotics were administered.

During the second week of December, 1953, at the time when the first wave of the outbreak was about at its peak, serological studies were started. These consisted of determination, against influenza virus, of the rise of antibody titers in serum samples, obtained successively from patients in acute and convalescent stages of the disease.

MATERIALS AND METHODS

For hemagglutination-inhibition tests the method described by the Expert Committee on Influenza of WHO in its first report, 1953, was employed with slight modification. The determinations were carried out on paired serum samples obtained successively from patients in acute and convalescent stages of the disease. All serum samples were stored at −20°C., until use. Prior to testing they were thawed and inactivated at 56°C. for 30 minutes. The influenza virus

TABLE 1

Results of serological studies on patients in an outbreak of influenza at the American University of Beirut during winter 1953–54

Patient	Acute and conv. serum samples: Date	Hemagglutination inhibition titers				Four-fold or more rise in titer to influenza viruses
		A/PR8/34	A/FW/1/50 (Cuppett)	B/Lee/40	C-1233	
KM	15/12/53	1/128	1/4	1/32	ND*	A-prime
	11/1/54	1/128	1/32	1/32	ND	
OM	15/12/53	1/64	1/16	1/8	ND	
	7/1/54	1/64	1/16	1/8	ND	0†
KF	15/12/53	1/64	1/8	1/8	1/16	A and A-prime
	12/1/54	1/512	1/128	1/8	1/16	
AHS	16/12/53	1/16	1/4	1/32	ND	A and A-prime
	7/1/54	1/64	1/64	1/32	ND	
AZ	19/12/53	1/32	1/4	1/16	1/16	A and A-prime
	9/1/54	1/512	1/256	1/16	1/16	
YB	28/12/53	1/256	1/8	1/16	1/16	
	12/1/54	1/256	1/8	1/32	1/16	0
SK	30/12/53	1/16	1/8	1/32	ND	
	20/1/54	1/16	1/8	1/32	ND	0
MS	2/1/54	1/32	1/4	1/2	1/16	
	28/1/54	1/32	1/4	1/2	1/16	0
EF	8/1/54	1/64	1/4	1/4	ND	
	28/1/54	1/64	1/4	1/4	ND	0
FK	8/1/54	1/64	1/4	1/32	1/32	
	28/1/54	1/64	1/4	1/512	1/32	B
NS	8/1/54	1/32	1/4	1/4	1/16	
	28/1/54	1/32	1/4	1/32	1/16	B
WM	14/1/54	1/64	1/8	1/4	ND	
	10/2/54	1/64	1/8	1/32	ND	B
JM	19/1/54	1/64	1/16	1/8	ND	
	10/2/54	1/64	1/16	1/8	ND	0
GB	19/1/54	1/64	1/8	1/16	1/32	
	10/2/54	1/64	1/8	1/16	1/32	0
AF	25/1/54	1/64	1/16	1/16	ND	
	25/2/54	1/64	1/16	1/128	ND	B
HH	25/1/54	1/64	1/32	1/4	1/32	
	20/2/54	1/64	1/64	1/16	1/32	B
AD	25/1/54	1/32	1/8	1/8	1/16	
	20/2/54	1/32	1/8	1/8	1/16	0
HA	26/1/54	1/16	1/8	1/4	ND	
	23/3/54	1/16	1/8	1/4	ND	0

* ND = Not done.

† 0 = No rise in titer; or rise in titer less than four-fold.

antigens employed for these tests were PR_8 strain of type A, FW/1/50 (Cuppett) strain of type A-prime, Lee strain of type B,[3] and 1233 strain of type C[4].

[3] PR_8, FW/1/50 (Cuppett), and Lee influenza virus antigens were kindly supplied by Dr. Morris Schaeffer of the Virus and Rickettsia Section, Communicable Disease Center, Montgomery, Alabama. These antigens were sent to us in lyophilized state.

[4] 1233 strain of influenza C virus was received through the courtesy of Dr. Richard Taylor of the NAMRU Laboratories, Cairo, Egypt.

Two-fold dilutions of each serum sample were carried out using 0.85 per cent saline as the diluent. Equal amounts, 0.25 ml., of each dilution of serum and antigen, containing 4 hemagglutinating units, were mixed. To the tubes, containing this serum-virus mixture, 0.5 ml. of 0.5 per cent washed chick erythrocytes was next added. The same amount of cells was also added to a control tube containing 0.5 ml. of normal saline. The tubes were shaken well and incubated at room temperature (22° or 24°C.) for 75 minutes, after which the titration end point was determined on the basis of the pattern of settled cells. Because influenza C virus rapidly elutes from red cells at room temperature, the hemagglutination-inhibition tests, when this virus was employed as antigen, were carried out in the cold, and the tests were read after 90 minutes. In either case the titer was expressed as the highest initial dilution of serum which effected complete inhibition of agglutination. Employing this method 18 paired serum samples, obtained successively from patients in acute and convalescent stages of disease, were tested against each of influenza A, A-prime and B virus antigens, and some against influenza C virus also.

The results of these determinations are given in Table 1.

RESULTS AND DISCUSSION

Lennette and his co-workers (1941) reported that, in an epidemic, influenza A and B viruses might occur at the same time in the same population and even in the same individual. Similar observations have also been made by Hare *et al.* (1943), and others (Kilbourne *et al.*, 1951; Burnet *et al.*, 1946; Commission on Acute Respiratory Diseases, 1948). Concurrent infections of children during the spring of 1947 with influenza A-prime and C viruses have also been reported by Francis *et al.* (1950).

An analysis of the data given in Fig. 1 and Table 1, makes it apparent at once that an outbreak of influenza, which manifested itself in two distinct waves, had occurred. It is of interest to note that there has been close chronological correlation between (a) the periods in which the two waves of the outbreak appeared, and (b) the time in which positive evidence for the presence of influenza A-prime, A and B viruses was supplied by serological tests. It can also be seen that 1 of 9 patients in the first wave showed a 4-fold or more rise of antibody titer in his blood when tested with influenza A-prime virus, and 3 of 9 individuals in the same group showed a simultaneous increase of antibody titer to both influenza A-prime and A viruses. Furthermore, 5 of 9 patients in the second wave showed a similar rise of titer when tested with influenza B virus. While rise in titer to both influenza A and A-prime viruses may suggest the possibility of mixed infections with these two viruses, it may indicate as well a current infection with influenza A-prime virus alone. A rise in titer of antibodies to influenza A virus may occur in patients infected with influenza A-prime virus provided they have had previous experience with the former virus (Davenport *et al.*, 1953). Thus, it is possible that the rise of antibody titer in serums of the three patients, in the first wave of the outbreak, to influenza A virus, was due to this type of an anamnestic reaction.

SUMMARY

An outbreak of influenza occurred among students and employees at the American University of Beirut during the winter season of 1953–54. It appeared in two distinct waves. The first wave, started in the last week of November and reached its peak in the second week of December, 1953, to decline thereafter. The second wave followed the first wave within two or three weeks showed a sharp rise, and disappeared during the first week of February. Hemagglutination-inhibition tests, using as antigens influenza A, A-prime, B and C viruses, were carried out on a limited number of paired serum samples obtained successively from patients in acute and convalescent stages of the disease. The results of these determinations indicated that 1 of 9 patients in the first wave had a 4-fold or more rise of antibody titer in his blood when tested with influenza A-prime virus, and 3 of 9 individuals in the same group had a simultaneous rise of antibody titer against both influenza A-prime and A viruses. Furthermore, 5 of 9 patients in the second wave showed a similar rise of titer in their blood when tested with influenza B virus.

REFERENCES

Burnet, F. M., Stone, J. D., and Anderson, S. G., 1946. An epidemic of influenza B in Australia, *Lancet* **1**: 807–811.

Commission on Acute Respiratory Diseases, 1948. Studies of the 1943 epidemic of influenza A. IV. The antibody response to infection, *Am. J. Hygiene* **48**: 287–291.

Davenport, F. M., Hennessy, A. V., and Francis, T., Jr., 1953. Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus, *J. Exp. Med.* **98**: 641–655.

Francis, T., Jr., Quilligan, J. J., Jr., and Minuse, E., 1950. Identification of another epidemic respiratory disease, *Science* **112**: 495–497.

Hare, R., Hamilton, J., and Feasby, W. R., 1943. Influenza and similar respiratory infections in a military camp over a period of three years, *Canad. J. Pub. Health* **34**: 453–464.

Kilbourne, E. D., Anderson, H. C., and Horsfall, F. L., 1951. Concurrent infection with influenza A and B viruses in a single epidemic of influenza, *J. Immunol.* **67**: 547–558.

Lennette, E. H., Rickard, E. R., Hirst, G. K., and Horsfall, F. L., Jr., 1941. The Diverse Etiology of Epidemic Influenza, *Pub. Health Rep.* **56**: 1777–1788.

World Health Organization, 1953. Technical Reports Series Number 64.

HOST-VIRUS RELATIONS IN ENCEPHALOMYOCARDITIS (EMC) VIRUS INFECTIONS

I. Infections of Wild Rats

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Encephalomyocarditis (EMC) infections were endemic in the vicinity of the Virus Research Institute at Entebbe from 1946 to 1951 (Dick, 1953). Actual isolations of the agent, at that time referred to as Mengo encephalomyelitis, were made from rhesus monkeys and a baboon caged out-of-doors, as well as from mosquitoes (*Taeniorhynchus* (*Coquillettidia*) *fuscopennatus*) caught in the same monkey runs. Additional isolations were obtained from a mongoose (*Ichneumia albicuada ibeana*) and a laboratory worker. Serologic surveys indicated that the disease was well disseminated locally. Animals with neutralizing antibodies of natural occurrence included rhesus monkeys and 2 species of wild rats, the black rat (*Rattus rattus kijabius*) and the multimammate rat (*Mastomys natalensis ugandae*). Since 1952 there has been no indication that EMC virus has been active in the area. The purpose of present investigations was to study pathogenesis and possible modes of transmission of EMC virus infections among rats and mongooses which, on the basis of previous experience at Entebbe, are considered to be natural hosts. Studies of EMC infections in mongooses are presented in the following communication (Kilham, Mason, and Davies, 1955b). Mosquito transmission of EMC virus was studied by means of hemocele inoculations of *T. fuscopennatus*. It is hoped that these studies will contribute to an understanding of how the infection may maintain itself in nature.

METHODS

Methods of preparing tissues, isolation of virus, inoculation of animals, and performance of serum neutralization tests, are described in a previous communication (Kilham *et al.*, 1955a). It may be repeated that 2 strains of EMC virus originally isolated at Entebbe, one from a baboon and one from a rhesus monkey, were used for study. Antiserum prepared against a strain of EMC virus originally isolated in Dania, Florida (Helwig and Schmidt, 1945) was used in neutralization tests to check identity of viruses isolated from inoculated animals. Unless otherwise stated, passages were made by intraperitoneal inoculation of animals with 0.1 ml. of a 1:10 tissue suspension in meat infusion broth. Throughout this paper titers are expressed as dilutions containing one mouse LD_{50} per 0.03 ml.

[1] Work done while on excused absence from the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute. Present address, Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

TABLE 1

Titers of virus in various tissues of black rats inoculated with EMC virus intraperitoneally

Rat	Age inoc. (days)	Days after inoculation	Condition	Blood	Brain	Fem. mus.	Heart	Spleen	Ileum	Feces	Urine
199R	14	4	Paralyzed	3.5*	7.3	4.7	4.7	3.5	<1	None	None
198RA	15	8	Paralyzed (one leg)	None†	None	2.5	—	1.5	None	None	—
198RB	15	6	Well	None	2.3	1.0	None	<1	None	None	—
169R	19	4	Well	—	3.0	2.3	—	—	—	—	—

* Titers expressed as inverse log of dilution killing 50% of mice inoculated.

† None: No virus isolated from 1:10 suspension.

Animals. Wild animals investigated were live-trapped in and near Entebbe. Those of main interest were the black rat, multimammate rat, and 3 species of field rats: *Lophuromys aquilis laticeps*, *Tatera liodon smithi*, and *Arvicanthis abyssinicus nubilans*.

Mosquitoes. T. fuscopennatus which were freshly caught on the evening before use, received virus inoculations by the method of Weathersby (1952). They were then maintained on raisins in individual glass tubes. When ground for titration, 10 mosquitoes per ml. of infusion broth were considered equivalent to a dilution of 10^{-2}.

RESULTS

Black rats. Black rats were found to be relatively resistant to infection with EMC virus. Overt infections were induced only in suckling rats. Two litters, born in captivity from nonimmune mothers, were inoculated with infectious albino rat brain suspensions, representing 10th and 11th animal passages. The first litter of 5 black rats, inoculated when 2 weeks old, was helpless within 4 days. Titrations of tissues from one rat (199R, Table 1) gave titers of $10^{-7.3}$ for brain, $10^{-4.7}$ for both femoral muscle and heart, and of $10^{-3.5}$ for blood. In a second litter of 6 rats, 15 days old, all remained well except for one (198RA, Table 1) which had paralysis of one hind leg 8 days later. Femoral muscle of this rat had a titer of $10^{-2.5}$ but virus could not be recovered from the brain. There were histologic changes consistent with myositis in muscle of the affected leg. These changes included destruction of fibers and infiltration with lymphocytes, monocytes, and a few polymorphonuclear leucocytes. No microscopic lesions were observed in the brain. A litter mate (198RB) sacrificed when well 6 days after inoculation, had a virus titer of $10^{-2.3}$ in brain. No virus was recovered from a suspension of blood. There was, therefore, evidence that virus may have proliferated in sucklings of this litter without development of apparent illness. A similar situation was observed in a third litter of 4, inoculated when 19 days old. Although none became ill, brain from one rat, harvested 4 days after inoculation, had a virus titer of 10^{-3} (169R, Table 1). Pooled serum from the 3 remaining rats, taken 3 weeks after inoculation, neutralized over 100 LD_{50} of virus.

Attempts to infect adult black rats and to induce an intestinal carrier state. Attempts to produce apparent illness in adult, nonimmune black rats were generally unsuccessful. However, virus was given to adults by several routes to determine whether an intestinal carrier state might be induced. In a preliminary experiment 6 rats were each fed an infected mouse carcass. Two rats were sacrificed in 24 hours. EMC virus was readily recovered from rectal feces of both but from neither contents nor wall of the ileum. Three rats, sacrificed when well at intervals of 4, 6, and 9 days, had no demonstrable virus in suspensions of feces, ileums, or spleens. Rectal feces from an additional rat, killed when well 6 days after the original feeding, had a virus titer of $10^{-1.7}$. No virus, however was recovered from the wall of the ileum

In a second series of experiments, mouse carcasses were fed daily. One rat, sacrificed after feeding 4 days on infected mice, had titers of $10^{-2.5}$ in feces and of $10^{-4.5}$ in the wall of the ileum, in addition to low titers in femoral muscle and spleen. Three other rats, fed continuously from 5 to 9 days on paralyzed mice, had no demonstrable virus in gut or feces when sacrificed 4 days to 3 weeks after their last infective meal.

Dick (1953) has reported recovery of EMC virus from feces and/or urine of a few black rats, killed when well 15 days after parenteral inoculation. In an attempt to repeat these experiments, 3 groups of black rats ranging from 35 to 115 gm. were inoculated. The first group of 2 rats received a 1:10 suspension of 8th passage albino rat brain intracerebrally. Both remained well and neither had demonstrable virus in brain, ileum, or feces when sacrificed 16 days post inoculation. A second group of 2 rats was inoculated intraperitoneally with a 1:10 suspension of infected brain from a suckling black rat. One rat sacrificed when well at 11 days had no demonstrable virus in feces and minimal amounts in suspensions of ileum and of spleen. No virus was recovered from a second rat killed at 13 days. A final group of 4 adults and one juvenile rat received intraperitoneal inoculations of virus which had been previously passed in multimammate rats. The juvenile rat, which weighed 47 gm., was killed when well at 4 days. No virus was recovered from blood, brain, or muscle. Spleen, however, had a titer of 10^{-1}, ileum of $10^{-1.5}$, and feces of $10^{-1.5}$ or more. A second rat, weighing 103 gm. was also sacrificed at 4 days. The same tissues as in the preceding rat, plus urine and feces, were assayed, but results were entirely negative. Remaining adults were sacrificed in good health at 2 weeks. None of the 3 had demonstrable virus in ileum or in fecal suspensions.

Multimammate rats. Eight successive passages of EMC virus were carried out in multimammate rats by intraperitoneal inoculation of 0.1 ml. of 1:10 suspensions of infectious muscle or spleen. Strain of virus was one originally isolated from a rhesus monkey. Table 2 shows pattern of virus distribution throughout the body. Rat 141 was studied when well and presumably prior to illness. Four days after inoculation, virus was recovered in appreciable quantity only from the intestinal tract, the ileum having a titer of $10^{-2.3}$ and feces of $10^{-1.3}$. In rats dead or moribund from disease, there was generally evidence of viremia. Results did not indicate, however, that recovery of virus from various

TABLE 2

Titers of virus in various organs of multimammate rats infected with EMC virus

Rat No.	Exposure to virus	Days after exposure	Condition	Blood	Brain	Fem. mus.	Heart	Spleen	Ileum	Feces	Other
138	I.p.	4	Moribund	1.7*	5	3.7	<3.5	3.7	4.2	4.6	Tapeworm, <1
200	I.p.	4	Dead	4	1.5	1.0	—	3.3	3.7	2.5	Urine, none
141	I.p.	4	Well	None†	<1	None	None	None	2.3	1.3	Tapeworm, none
207	I.p.	6	Dead	1.7	5.8	2.7	4.5	3.5	3	3	—
193	Oral	5	Dead	6	4.7	4	—	—	6.2	4.5	Urine, 3.5
233	Oral	5	Well	3.5	2.7	2	—	4.5	2.7	1.8	Bladder, 3.5
234	Oral	6	Moribund	None‡	6	3.5	3.3	3.5	—	—	Bladder, 2.5; diaphragm, 2.7

* Titers expressed as inverse log of dilution killing 50% of mice inoculated.

† None: No virus isolated from 1:10 suspension.

‡ Tested at 10^{-2} only.

organs was an effect of virus present in the residual blood they contained when harvested. Thus in rats 138 and 207, virus content was actually lower in blood than in any of the tissues tested.

A question of interest was what type of damage was responsible for death. Encephalitis and myocarditis appeared to be of special importance. In 3 rats (nos. 138, 207, 234, Table 2) brain tissue yielded highest titers of virus. Histologic sections of the brain made on one rat (207) revealed scattered foci of degeneration and cellular infiltration with lymphocytes and macrophages. However, findings in other rats suggested that encephalitis may have been of lesser importance. For example, rat 200 (Table 2), well and active 4 days after inoculation, was found dead 5 hours later. No signs of paralysis had been observed and brain had a comparatively low virus titer of $10^{-1.5}$. Histologic sections gave no indication of lesions in brain or in femoral muscle. Unfortunately, the heart, preserved in formalin, was not titrated. Histopathologic examination of the myocardium, however, revealed diffuse infiltration with lymphocytes, monocytes, and a few polymorphonuclear leucocytes. There was no obvious muscle destruction. Congestion of lungs and liver was a finding which, in this rat, contributed to a diagnosis of acute congestive heart failure. Sections from the myocardium of rat 207 revealed a more severe myocarditis with destruction of muscle fibers. Multimammate rats usually became ill and died suddenly, so that other than extreme helplessness, no clinical signs were observed.

A point of interest in regard to virus transmission was the amounts of virus in ileum and rectal feces of multimammate rats receiving intraperitoneal inoculations (Table 2). Titers of the ileum were usually higher than those of rectal feces. The question arose as to whether infection could be passed by the oral-intestinal

TABLE 3

Titers of virus in various organs of field rats of 3 different genera inoculated with EMC virus intraperitoneally

Genus	Days after i.p. inoculation	Condition	Blood	Brain	Fem. mus.	Heart	Spleen	Ileum	Feces	Pleural Fluid
Avicanthis	3	Dead	4.3*	1.5	1.5	7.3	2.7	3	3.5	3.5
Lophuromys	5	Dead	2.0	5.5	4.2	6	1.7	3.5	<1	—
Tatera	4	Dead	5.4	3.5	2.0	6.3	5.8	4	1.8	<5.5

* Titers expressed as inverse log of dilution killing 50% of mice inoculated.

route. Accordingly, 3 multimammate rats were exposed to EMC virus by feeding them infected mouse carcasses, either for 2 days only (rats 233 and 234, Table 2) or continuously (rat 193). Course of illness and general distribution of virus in these rats did not differ significantly from those described above for rats inoculated intraperitoneally. Of interest, however, was the amount of virus recovered from the urine of rat 193 after death from disease. This animal had an unusually high viremia. The urine was free of blood macroscopically. Urinary bladders harvested from the 2 remaining rats were found to contain virus in titers as high or higher than those found in blood of the same animals. A survey of titers found in muscle tissues of various origin (Table 2) indicated that EMC virus probably proliferates in femoral muscles, as well as in the urinary bladders and diaphragm of infected multimammate rats.

Field rats. A few species of field rats were investigated for comparative purposes. Table 3 presents findings on 3 rats of different genera which were dead or moribund 3–5 days after intraperitoneal inoculation of virus. Two of the rats, *Arvicanthis* and *Tatera*, had straw-colored fluid in abdominal and in both pleural cavities. Highest titers of virus were encountered in the myocardium of all 3 rats. Damage to the myocardium, shown on histologic sections from *Arvicanthis* and *Lophuromys*, was similar to that described above for multimammate rats. Although 10 per cent tissue suspensions used for inoculation of these animals differed, this did not appear to have affected the general disease picture. Thus *Arvicanthis* and *Lophuromys* received third passage mongoose heart, and *Tatera*, splenic tissue from 7th passage of virus in multimammate rats. General distribution of virus in these rats, including presence of agent in intestinal walls and in feces, was similar to findings reported above.

Mosquitoes. EMC virus had been isolated in previous years from *T. fuscopennatus* collected within the Institute compound. To investigate one phase of their potentialities as vectors, namely, whether virus could multiply in them, mosquitoes were inoculated into the thorax with 1:10 brain suspensions of mice infected with the rhesus strain of EMC virus. At various intervals groups of 5 individuals were ground in a mortar. Titrations in mice of such groups, in one typical experiment, gave the following results: At 7 hours after inoculation the titer was $10^{-5.5}$, at 24 hours 10^{-6}, at 2 weeks 10^{-3}, and at 23 days 10^{-1}. Repeated

experiments with the same species as well as with *Aedes aegypti* gave equivalent results. Attempts to effect transmission by means of inoculated mosquitoes were negative in adult and suckling albino rats and mice.

DISCUSSION

A study of black rats was undertaken on a hypothesis that these rodents, as natural carriers of EMC virus, may spread infections by means of excreta. At the time Dick (1953) made this interesting speculation, he stated that obviously "more quantitative studies on the excretion of virus by rats" were needed. In our experiments we have not succeeded to any extent in inducing a true intestinal carrier state in black rats, either by feeding or by parenteral inoculation of EMC virus. Ross, in experiments reported by Haddow (1952) was likewise unsuccessful. He inoculated 3 black rats intraperitoneally, then tested urine and feces separately for 24 days. Virus was recovered from feces of one rat on the 17th day only. It would seem that there should be evidence of virus growth in intestinal wall or elsewhere in the body, for a significant carrier state to exist. A few black rats in our experiments did have low titers of virus in feces and visceral organs for as long as 4 days after exposure to virus. Occasional recovery of EMC virus from rat feces several days or more after exposure could result either from the original feeding directly or from re-ingestion of virus from feces voided after the infective meal. An intestinal carrier state was not induced in albino rats in the course of similar experiments (Kilham *et al.*, 1955a). Thus, of 18 weanling and adult rats, most of which were actually paralyzed due to EMC virus, no virus was recovered from suspensions of gut or feces. These animals were killed 5 to 6 days after inoculation, except for 5 sacrificed between 11 and 27 days. These latter findings appeared of interest since, in southern United States, Warren *et al.* (1949) found that a high percentage of *Rattus norvegicus*, the wild progenitor of albino rats, had neutralizing antibodies against EMC virus. Norway rats should, therefore, also be considered as possible carriers. Results of surveys among wild mammals at Entebbe by Dick and Ross, summarized by Haddow (1952) showed that 20 of 82 sera obtained from black rats contained specific neutralizing antibodies. In 1954–55, however, only 4 of 58 black rats tested had antibodies, all in questionably low titer (3 sera neutralized 10 mouse LD_{50}'s and one serum 100 LD_{50}'s of virus). There was thus no definite evidence that EMC virus was active in the area at the time of present investigations. A hypothesis is offered that neither *Rattus rattus* nor *Rattus norvegicus* play much part in transmission of EMC virus. Being resistant to this agent, they presumably survive infections. Thus, when serological surveys are made, many of them are found to have neutralizing antibodies. From this point of view rats of the genus *Rattus* serve as indicator hosts for presence of EMC virus infections.

The situation with field rats, of which the multimammate was studied most extensively, appeared to be different. Adults were susceptible to infection, either by ingestion or by parenteral inoculation of EMC virus; furthermore, feces and the wall of the ileum contain large amounts of virus. Because virus is present in blood and apparently throughout many tissues, carcasses of such rats

might serve as sources of infection, whether consumed by some predator or by another rat. Such considerations suggest that field rats are potentially important reservoirs and transmitters of EMC virus in nature. Because they are highly susceptible, few individuals may survive to have neutralizing antibodies. The fact that EMC virus can infect a wide spectrum of mammalian hosts, probably favors its survival.

Results of the hemocele inoculation experiments suggest that recovery of EMC virus from wild caught *T. fuscopennatus* was no indication that virus can proliferate in and be transmitted by such mosquitoes. One may presume that the infected wild caught mosquitoes had recently fed on monkeys which had acquired spontaneous infections in outdoor cages. Carriage of virus may thus have been purely mechanical. Dick (1953) was unable to transmit EMC virus by means of mosquitoes which had fed on infected monkeys. Gillett, who cooperated in these experiments (personal communication, 1955), found that *T. fuscopennatus* could pick up virus from infected monkey blood and retain it passively for as long as 4 days.

EMC virus has the peculiarity of inducing chiefly an encephalitis in albino rats (Kilham *et al.*, 1955a) and a myocarditis in field rats. In this respect infections in field rats resembled more closely those described for mongooses in a following communication. Rats were not submitted to cardiac puncture prior to inoculation of virus. There was, thus, no question of what effect this procedure might have had on a subsequent myocarditis.

SUMMARY

1. Black rats (*Rattus rattus*), except when in the suckling stage, have proved resistant to infection with EMC virus. No continuous intestinal carrier state could be induced in them.

2. Multimammate (*Mastomys coucha*) and other field rats proved highly susceptible to EMC virus infections. Virus was recovered from feces, intestinal wall, blood, and a variety of organs from bodies of infected animals. A hypothesis is offered that these small mammals may be of principal importance in maintaining the disease in nature.

3. Field rats inoculated with EMC virus appeared to die principally from myocarditis.

4. Hemocele inoculations of EMC virus into *Taenyorhynchus fuscopennatus*, gave no indication that these mosquitoes are potential vectors.

REFERENCES

Dick, G. W. A., 1953. Epidemiological notes on some viruses isolated in Uganda, *Trans. Roy. Soc. Trop. Med. & Hyg.* **47**: 13–43.

Haddow, A. J., 1952. Mengo encephalomyelitis virus, *Ann. Rep. Vir. Res. Inst. Entebbe.* No. 3: 14–15.

Helwig, F. C., and Schmidt, E. C. H., 1945. A filter-passing agent producing interstitial myocarditis in anthropoid apes and small mammals, *Science* **102**: 31–33.

Kilham, L., Mason, P., and Davies, J. N. P., 1955a. Pathogenesis of fatal encephalo-

myocarditis (EMC) virus infections in albino rats, *Proc. Soc. Exp. Biol. and Med.* **90**: 383–387.

KILHAM, L., MASON, P., AND DAVIES, J. N. P., 1955b. Host-virus relations in encephalomyocarditis (EMC) virus infections. II. Myocarditis in mongooses, *Am. J. Trop. Med. & Hyg.* **5**: 655–663.

WARREN, J., RUSS, S. B., AND JEFFRIES, H., 1949. Neutralizing antibody against viruses of the encephalomyocarditis group in the sera of wild rats, *Proc. Soc. Exper. Biol. & Med.* **71**: 376–378.

WEATHERSBY, A. B., 1952. The role of the stomach wall in the exogenous development of *Plasmodium gallinaceum* as studied by means of haemocoel injections of susceptible and refractory mosquitoes, *J. Infect. Dis.* **91**: 198–205.

HOST-VIRUS RELATIONS IN ENCEPHALOMYOCARDITIS (EMC) VIRUS INFECTIONS

II. Myocarditis in Mongooses

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The present communication on encephalomyocarditis virus infections in mongooses is part of a study of the pathogenesis and transmission of EMC virus infections in natural hosts. Feral mongooses were apparently hosts of EMC virus in years when this agent was causing infections among wild rats and caged monkeys in the vicinity of Entebbe (Dick, 1953). Virus was isolated from the serum of 1 mongoose (*Ichneumia albicauda ibeana*) and serum from 1 of 4 mongooses tested was found to contain neutralizing antibodies. Fulminating myocarditis has been a striking feature of experimental infections in mongooses. Studies of EMC virus infections in an African grivet monkey (*Cercopithecus aethiops centralis*) and attempted infections in genets have been included for comparative purposes.

METHODS

Methods used have been presented elsewhere (Kilham *et al.*, 1955a). The strain of EMC virus used was one originally isolated from a baboon which had died at the Virus Research Institute. This strain had been carried through 6 intracerebral passages in mice. Tissues for histologic section were preserved in 10 per cent formalin.

Wild animals investigated were live-trapped in and near Entebbe. Those of main interest were 2 species of mongooses: the ichneumon (*Ichneumia albicauda*) and the banded mongoose (*Mungos mungo macrurus*). Palm civets (*Nandinia binotata arborea*) and genets (*Genetta tigrina stuhlmanni*) were also inoculated.

RESULTS

Mongooses. Both species of mongooses reacted in similar fashion to infection with EMC virus, whether exposure was by ingestion or by parenteral inoculation. They are, therefore, considered together in the present experiments. All inoculations were intraperitoneal and for 6 animals consisted of approximately 10^6 to 10^7 mouse LD_{50}'s of the baboon strain of virus in mouse brain suspension. Four continuous passages were carried out in ichneumons. Ten per cent suspensions of infected heart muscle, given intraperitoneally and containing approximately 10^4 to 10^5 mouse LD_{50}'s of virus were used for the last 3 passages. No

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TABLE 1

Titers of virus in various tissues and other specimens from mongooses and a grivet monkey which had died following infection with EMC virus

Animal	No.	Route inoc.	Days until death	Blood clot	Brain	Fem. mus.	Heart	Spleen	Gut	Feces	Fluid		Other
											Pleural	Pericardial	
Banded mongoose	1310	I.p.	3	1.6*	<1	1.4	3.8	3.3	2.5	—	2.5	—	Porocephelus 2.3
	1693	Oral	6	2.3	3.3	3.0	7.0	4.7	3.0	5.6	4.2	—	Urine none
Ichneumon	4	I.p.	4	2.7	1.5	1.0	5.5	3.5	2.8	2.5	5.2	—	—
	5	Oral	6	1.5	2.2	1.5	4.2	2.7	1.2	3.5	2.8	—	—
	9	I.p.	5	4.6	2.5	2.5	6	4.2	3.2	None†	—	6.5	—
Grivet monkey	1104	I.p.	5 (moribund)	3.5	2.5	3	—	3.5	>2.5	2.5	—	5.5	—

* Titers expressed as inverse log of dilution killing 50% of mice inoculated.
† None = No virus isolated from 1:10 suspension.

change of virulence in the course of these passages was observed. Two ichneumons and 2 banded mongooses became infected after ingestion of infected mouse carcasses. Of animals infected either by feeding or by intraperitoneal inoculation, 7 of 9 ichneumons and 3 of 5 banded mongooses died during the night 2½ to 6 days after exposure. The 6-day incubation was encountered in 2 mongooses, one of each species, which had fed on infected mice (Table 1). Since most mongooses died suddenly, signs of illness other than terminal lack of appetite were seldom observed. One mongoose died under observation. It had marked dyspnea and a whitish froth in nose and mouth. When picked up after death, straw-colored fluid, presumably from the lungs, poured from its mouth. One ichneumon and 2 banded mongooses which remained well developed neutralizing antibodies following exposure to EMC virus either intraperitoneally (banded mongooses) or by ingestion of mouse carcasses (ichneumon).

Straw-colored fluid in pleural and/or pericardial cavities was the principal gross abnormality found in 8 of 9 mongooses which had died of infection. Three of the 8 also had pulmonary edema of such degree as to be readily apparent. A few animals had small amounts of fluid in the abdominal cavity. No free hemorrhages, petechiae, pulmonary consolidation or other evidences of disease were noted. Table 1 presents results of virus titrations of various organs from mongooses which had become ill or had died of EMC virus infections. Regardless of how virus was given, the myocardium contained the greatest concentration of virus. For example, in banded mongoose 1693, the virus titer of heart muscle was 10^{-7}, of brain $10^{-3.3}$, and of blood $10^{-2.3}$. Ichneumon 9 was the only mongoose from which clear pericardial fluid was obtained. This fluid had a titer of $10^{-6.5}$ and the heart muscle of 10^{-6}. Titers of pleural fluid from all mongooses from which it was obtained, were higher than those in blood of the same animals.

A number of mongooses, illustrated by ichneumon 4 (Table 1), had a moderate amount of virus in feces even when inoculated intraperitoneally. Virus in rectal

feces was accompanied in all instances by recovery of virus from wall of the ileum.

An occasional mongoose of either genus harbored endoparasites identified as *Porocephalus armillatus*. These were about 1 cm. in length and were generally abundant near peritoneal surfaces and especially within the spleen. Specimens from 2 ill mongooses were dissected free of host tissue, washed in broth, and titrated. Parasites from banded mongoose 1310 (Table 1) had a titer of $10^{-2.3}$, a titer exceeding that encountered in blood, brain, or femoral muscle of the same animal. Parasites from an ichneumon had a titer of 10^{-1}.

Other animals. Two other types of wild carnivores, belonging to the same family (Viverridae), were resistant to infection with virus preparations fatal to mongooses. Of 6 genets and 2 palm civets exposed to EMC virus, half of them

FIG. 1. Transverse section of heart showing extensive fiber damage (pale areas) accompanying severe myocarditis in ichneumon 5. (P.T.H. × 4.2).

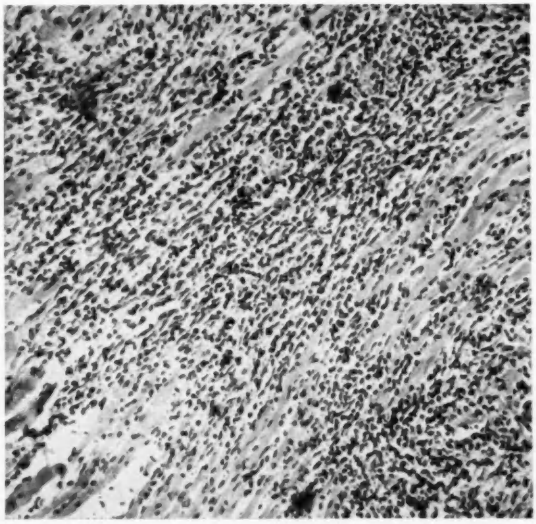

FIG. 2. Myocardium of ichneumon 4 showing gross infiltration with inflammatory cells. (H & E × 120).

by intraperitoneal inoculation and the others by feeding infected mice, none became ill. Furthermore, of 5 genets subsequently bled, 3 had failed to develop neutralizing antibodies following exposure.

An adult grivet monkey, studied for comparative purposes, was inoculated intraperitoneally with approximately 10^7 mouse LD_{50}'s of the rhesus strain of EMC virus. Four days later the monkey appeared well. Virus, however, was recovered from blood in a titer of $10^{-4.5}$ and from feces in a titer of 10^{-1}. The animal was moribund on the following day. On necropsy, presence of a small amount of pericardial fluid was the only gross abnormality noted. The heart, preserved entire for histologic sections was not titrated, but distribution of virus in other parts of the body is shown in Table 1. Virus titers were approximately the same as those encountered in mongooses, although viremia was more pronounced. Pericardial fluid had a titer of $10^{-5.5}$. As discussed below, histologic sections provided evidence of myocarditis.

Pathology. A myocarditis was observed in all infected mongooses. In some cases (Fig. 1) destruction of myocardial fibers was extensive and was accompanied by gross infiltration with inflammatory cells (Fig. 2). Lesions were found in all areas of the heart but were most severe in the ventricles. The cellular infiltrate contained some polymorphonuclear cells but was chiefly composed of lymphocytes and macrophages (Fig. 3). In some cases, the endocardium was damaged and heavily infiltrated with inflammatory cells (Figs. 4 and 5). Antemortem thrombus on the endocardium was sometimes present. In some cases there was little evidence of myocarditis other than small focal collections of

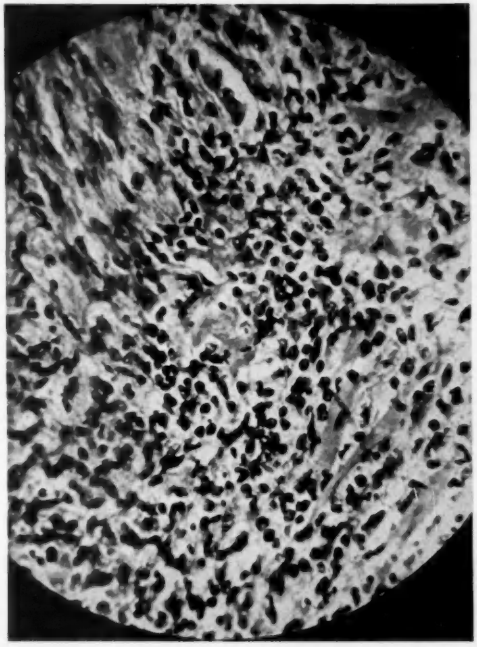

FIG. 3. Myocardium of ichneumon 4. Cellular infiltrate composed chiefly of lymphocytes and macrophages with some polymorphonuclear cells. (H & E × 375).

chronic inflammatory cells, diffusely scattered throughout the myocardium (Fig. 6).

No myositis was seen in sections of the striated muscles taken from 3 animals. Brains of 2 mongooses were examined; one was normal, whereas the other brain was affected with a mild encephalitis characterized by engorged blood vessels and some perivascular cuffing with polymorphonuclear leucocytes and lymphocytes. Small focal collections of these cells were present outside the blood vessel sheaths but there was no evidence of severe neurone damage. A moderately extensive interstitial myocarditis, similar to that seen in the mongoose heart, was found in a monkey (*C. aethiops*) which died of acute heart failure. Its distribution was patchy without any particular localization and cells in focal lesions were chiefly lymphocytes. No other tissues were examined.

Needle trauma to heart. A question of possible importance was whether cardiac puncture, performed before exposure to virus, predisposed animals to myocarditis. Of mongooses studied, 9 which became moribund or died had had cardiac puncture either on the day of or within a 3-day period prior to inoculation. Three other mongooses, which experienced inapparent infections, were bled 3, 6, and 22 days respectively prior to inoculation. Two ichneumons, however, were not bled. Both of them developed severe myocarditis subsequent to

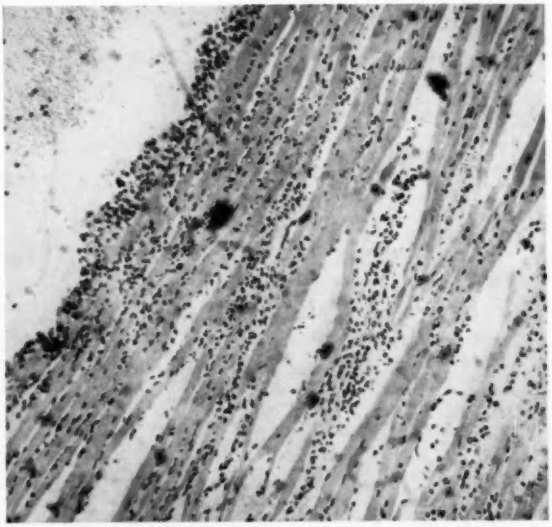

FIG. 4. Endocardium of ichneumon 4 showing heavy infiltration with inflammatory cells. (H & E × 120).

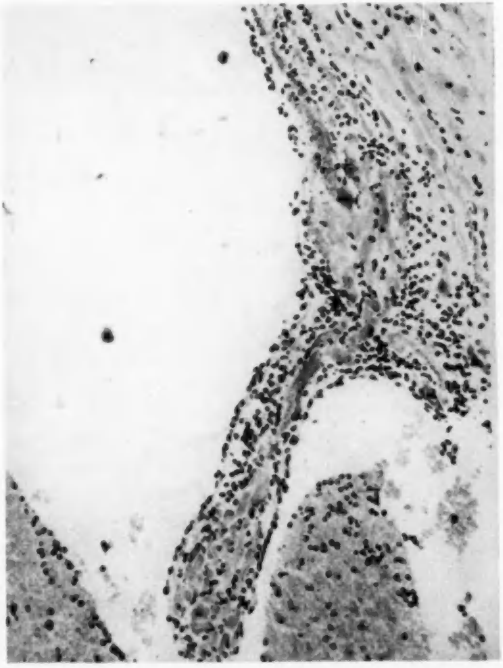

FIG. 5. Endocardium of banded mongoose 1693, showing damage and cellular infiltration. (H & E × 140).

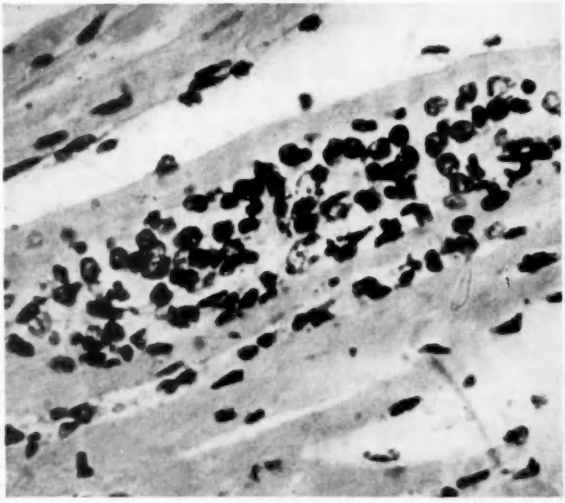

FIG. 6. Myocardium of banded mongoose 1693. Myocarditis in evidence only by small focal collections of chronic inflammatory cells. (H & E × 470).

inoculation. Of other animals submitted to cardiac puncture within a few days of exposure, the grivet monkey subsequently died, but 5 genets and 2 palm civets never became ill.

Neutralization tests. Serum specimens obtained from 7 ichneumons, 7 banded mongooses, 6 genets, and 2 palm civets, all freshly trapped, contained no demonstrable neutralizing antibodies against EMC virus.

DISCUSSION

A variety of mammals, suspected on the basis of previous investigations (Dick, 1953) of being natural hosts of EMC virus, have been studied in regard to their potentialities as transmitters of this agent in nature. Present experiments have shown that mongooses are susceptible to infection by the oral route. One may speculate that they could acquire natural infections by predation on field rats and possibly other small mammals carrying EMC virus. Recovery of the agent from rectal feces as well as from intestinal walls of mongooses infected by parenteral inoculation indicates that these animals could function as intestinal carriers. It was of additional interest to recover virus in good titer from an endoparasite encountered in an infected mongoose, *Porocephalus armillatus*. Since virus located within such an arthropod might well be protected from circulating antibodies of its mammalian host, a mechanism for latent survival is suggested. Unfortunately, mongooses were not available in sufficient numbers to pursue this problem. Natural infections of mongooses may well be less fatal than the fulminating disease induced by experimental exposure to large amounts of virus. In a limited serologic survey of wild mongooses and genets,

neutralization tests gave no indication that EMC infection had occurred in the Entebbe area during 1954 and 1955.

Mongooses inoculated with a strain of virus originally isolated in Uganda died suddenly and had gross and microscopic findings apparently similar to those reported for 2 chimpanzees and a gibbon which died of natural infections in Florida (Helwig and Schmidt, 1945; Schmidt, 1948). In all infected mongooses examined, the principal damage was to the heart with little histopathologic evidence of involvement of the central nervous system. Results of titrations demonstrated that heart muscle contained greater amounts of virus than other tissues tested. Mongooses almost always died at night, making it difficult to observe onset of illness. It is probable that, since mongooses are nocturnal animals, activity at this time may have precipitated death from acute cardiac failure in these carnivores. EMC virus was recovered in relatively high concentrations from pleural and pericardial fluids. Since these concentrations were higher than for blood, it is conceivable that virus came from another source. One possibility would be that virus is liberated directly into pericardial fluid by infected cells of the myocardium. Similarly, infected muscle cells of the diaphragm might liberate virus into pleural fluid. Unfortunately segments of diaphragm, although found positive in infected field rats (Kilham *et al.*, 1955b), were not tested in mongooses.

The question of whether cardiac puncture had any definite effect on precipitating or intensifying myocarditis due to EMC virus is of interest from several points of view. For one, Pearce (1939 and 1942) has shown that preceding damage, whether by direct trauma or by overburdening the heart, intensified lesions in the rabbit heart due to Virus III and a series of other viruses pathogenic for these animals. Thus, simple cardiac puncture 3 to 5 days prior to peripheral inoculation of Virus III markedly increased the incidence, extent, and severity of myocarditis lesions due to this agent. Second, one would like to know whether prior bleeding from the heart made experimental infections differ significantly from those of natural occurrence. The fact that 2 mongooses which were not bled died of fulminating myocarditis indicated that trauma was not essential to its inception.

SUMMARY

1. Mongooses of 2 species (*Ichneumia albicauda* and *Mungos mungo*), exposed to EMC virus either by the oral route or by parenteral inoculation, have proved to be extremely susceptible to a severe myocarditis.

2. The role which these animals may play in maintaining EMC virus in nature, either by predation on other mammals or by becoming intestinal carriers, is discussed in relation to experimental data.

3. An endoparasite commonly found in mongooses, *Porocephalus armillatus*, was found to contain virus when recovered from infected animals.

4. Although the effects of cardiac puncture are an important consideration in animals developing myocarditis under experimental conditions, mongooses not submitted to this procedure developed a myocarditis equal in severity to those which had been bled from the heart prior to inoculation.

REFERENCES

DICK, G. W. A., 1953. Epidemiological notes on some viruses isolated in Uganda, *Trans. Roy. Soc. Trop. Med. & Hyg.* **47**: 13–43.

HELWIG, F. C., AND SCHMIDT, E. C. H., 1945. A filter-passing agent producing interstitial myocarditis in anthropoid apes and small mammals, *Science* **102**: 31–33.

KILHAM, L., MASON, P., AND DAVIES, J. N. P., 1955a. Pathogenesis of fatal encephalomyocarditis (EMC) virus infections in albino rats, *Proc. Soc. Exp. Biol. & Med.* **90**: 383–387.

KILHAM, L., MASON, P., AND DAVIES, J. N. P., 1955b. Host-virus relations in encephalomyocarditis (EMC) virus infections. I. Infections of wild rats, *Am. J. Trop. Med. & Hyg.* **5**: 647–654.

PEARCE, J. M., 1939. Cardiac lesions in rabbits produced by a filtrable virus (virus III), *Arch. Path.* **28**: 827–845.

PEARCE, J. M., 1942. Susceptibility of the heart of the rabbit to specific infection in viral diseases, *Arch. Path.* **34**: 319–333.

SCHMIDT, E. C. H., 1948. Virus myocarditis: pathologic and experimental studies, *Am. J. Path.* **24**: 97–118.

THE CEREBELLO-EXTRAPYRAMIDAL FORM OF TRYPANOSOMIASIS

Report of a Case Simulating an Abiotrophy with Post-Mortem Study

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It is well known that juvenile general paresis may closely simulate the so-called abiotrophies of the central nervous system. Even in the adult, syphilis could be mistaken for a cerebellar atrophy were it not for such tell-tale evidence as the pupillary changes and the serological reaction. The same is true of some cases of African trypanosomiasis.

It is not the purpose of this paper to review the reported cases of cerebello-spastic and extrapyramidal forms of sleeping sickness. Several years ago, in reporting the case of a white man with sleeping sickness, one of the authors (L. v B.), working with Borremans (1933) reviewed the subject. The present report illustrates the diagnostic problem in a young colored male.

Case report. Shabani, a 14-year-old colored boy from the province of Kivu, Belgian Congo, was first brought to the hospital by his mother because of alleged mental retardation. He had been treated by two different physicians in the past for trypanosomiasis, but no further details about the illness or the treatment were available.

The father died in 1943 after an acute illness of unknown nature which lasted two days. The mother is living and well. After giving birth to Shabani, she is reported to have had several miscarriages. There are no living siblings.

The patient was 14 years old and had not yet reached puberty (Fig. 1). His height was 2 feet, 6 inches and he weighed 72 pounds. His abdomen, his mons veneris, and his upper thighs were unusually fat. The picture was reminiscent of that in the adiposogenital syndrome. In addition, there was bilateral cryptorchidism and gynecomastia.

When examined standing in the nude, it was evident that his right shoulder was held lower than his left. This was occasioned by a dorsal scoliosis with a convexity to the left. Although the pedal arch seemed flatter than normal in the standing position, when the boy was recumbent both feet seemed to have an unusually high arch. When he held his hands extended in pronation there was noticeable hyperextension of the distal phalanges as compared with the proximal.

The boy was unable to sit still. His arms moved constantly. There were frequent choreoathetotic movements of the hands and fingers, particularly of his left hand (Fig. 1). Moreover, his big toe frequently underwent hyperextension movements. His gait was peculiar in that it had features of both spinal and cerebellar ataxia. His knees often knocked together while he walked. His gait was abnormally wide-based.

The boy's heart and lungs were normal. His liver did not appear enlarged. The edge of the spleen was palpable at the level of the umbilicus. The conjunc-

FIG. 1. Shabani. Note the adiposity and genital atrophy and the kyphoscoliosis. The hand was photographed while it was undergoing an involuntary choreiform movement.

tivae were of normal color; there were no stigmas of avitaminosis. Blood pressure (arm) was 90 mm. Hg, systolic, respiration 20, and pulse 98 per minute.

Laboratory examination. The blood Wassermann was negative. A lumbar puncture performed May 12, 1954 revealed clear fluid with 29 lymphocytes and 50 mg. per cent of albumin. The Wassermann and Kahn tests were negative. Neither trypanosomes nor Mott cells were seen on direct smear.

Neurological examination. There was bilateral incoordination: the patient was unable to walk with his eyes closed, and if not supported, would fall. The Romberg test was, however, negative. The heel-to-knee test was well performed on the right but there was slight dysmetria. Dysdiadochokinesia was pronounced bilaterally. The Bárány test revealed dysmetria caused by segmental motor instability.

Muscle tone was everywhere equal. Muscle power seemed to be diminished for a boy of his age. Deep tendon reflexes were active and equal throughout except for the Achilles reflexes, which were hyperactive. Abdominal reflexes were present. Plantar reflexes were present but could not be interpreted. Sensory examination revealed no abnormalities: there was no astereognosis.

The cranial nerves were all normal. Visual acuity, pupillary reflexes and the optic fundi all were within normal limits.

Psychiatric examination, (by Pie Masumbuko). The boy was logorrheic; he did nothing but beg for money. He had had no formal schooling whatever since

he became ill during his first school year at the age of 11. He complained of back pain when bending as well as of dizziness. He was very suggestible and repeated everything that was said to him. His conversation lacked logical sequence. His attention span was short, and though he was able to repeat short sentences, his repetition of numbers was rather poor. Judgment was poor, but he had no delusional ideas. His affect was rather flat. He was generally indifferent and apathetic. In summary, the psychiatric examination supported the diagnosis of mental deficiency.

Course of the illness. Quite suddenly a febrile illness broke out. There were no overt clinical manifestations. No cause was uncovered. The boy died on May 31, 1954. An autopsy was performed 40 minutes after death.

Clinical summary. This boy, who at the age of 11 already seemed mentally retarded, was said to have had two episodes of febrile illness which contributed to the arrest of his somatic and psychic development. At the time of the examination the following pertinent signs were observed:

1. From the endocrinological standpoint, the patient seemed to present a type of adiposogenital syndrome with bilateral cryptorchidism.

2. From the neurological standpoint the picture was that of a combined athetotic syndrome and there was constant motor agitation, high-arched feet, and kyphoscoliosis. Tendon reflexes were active. No decision could be reached as to whether the plantar response was pathological or not.

3. From the psychiatric standpoint he had severe mental retardation.

In view of the high-arched feet, the kyphoscoliosis, and the ataxic and other cerebellar signs, one would be inclined to think of hereditary ataxia; the presence of bilateral choreoathetotic movements would not exclude this diagnosis, nor would the mental deficiency which is seen not infrequently in this condition.

Were it not for the lymphocytosis of the cerebral spinal fluid, the diagnosis of Marie's type of ataxia with oligophrenia would have been indicated. However, one could not ignore the lymphocytosis nor the two episodes of febrile illness in the past which, according to the mother, had definitely aggravated the patient's condition. Trypanosomiasis had, therefore, to be considered in the differential diagnosis.

Autopsy. Severe meningitis over the convexity of the brain was clearly evident. The brain as a whole was atrophied, though the infero-lateral portion of the temporal poles had suffered most. Vertico-transverse sections revealed that the atrophy involved both white and grey substances; it was pronounced at the level of the anterior horns of the ventricular system, and the caudate nuclei were as atrophic as in Huntington's chorea (Fig. 2A). All ventricles showed a compensatory hydrocephalus. The cerebellum and brain stem appeared unusually small; the medulla seemed atrophic, and the olives were abnormally prominent. Vertico-transverse sections of the cerebellum also revealed moderate atrophy of the white matter, but the dentate nuclei were particularly small. The optic chiasm was small. The cerebral peduncles appeared atrophied. The substantia nigra was normally pigmented.

Microscopic examination. Various regions of the cerebral cortex were examined.

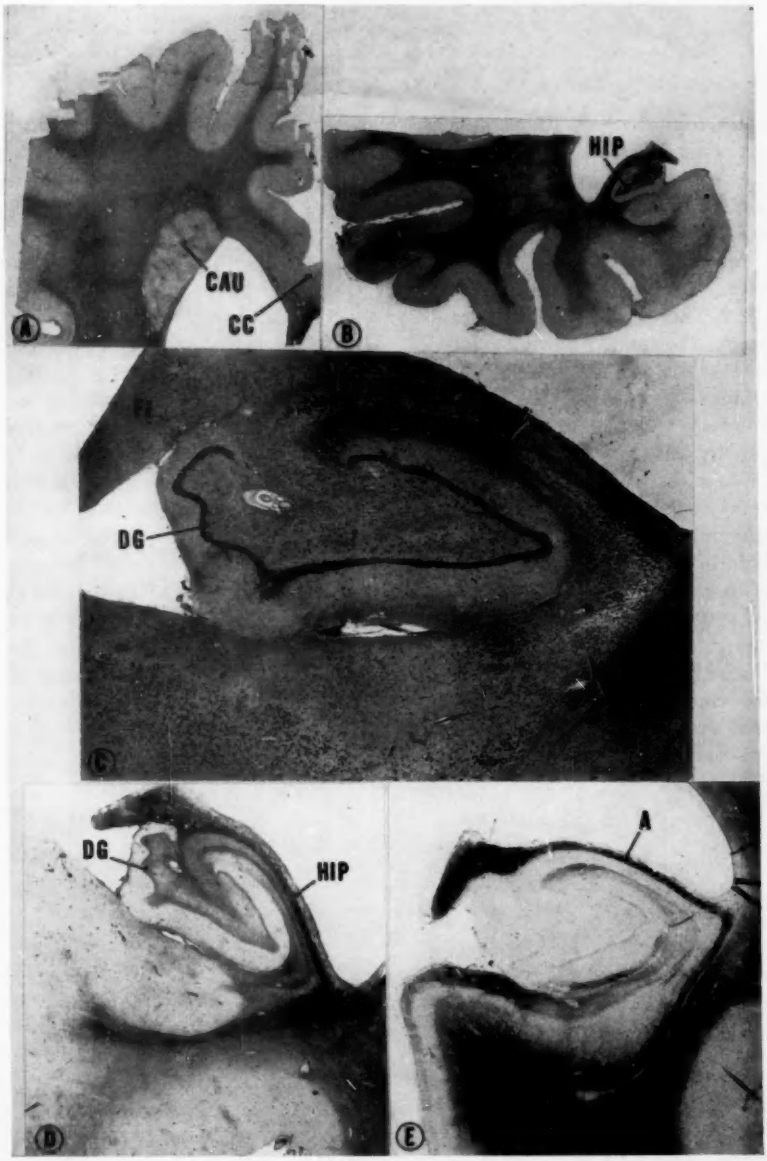

FIG. 2. A. Section through the frontal lobe, showing gliosis of the centrum ovale as indicated by the dark color. The head of the caudate nucleus (CAU) has the gnarled appearance characteristic of status marmoratus. The ventricle is enlarged. Holzer glial fiber stain. B. Gliosis of the white matter of the temporal lobe. The unstained islands in the white matter to the left and the larger unstained area in the gyrus to the right are sites of cell infiltrates. There is gliosis also of much of the hippocampus (HIP). Holzer stain. C. Cell loss and sponginess in the pyramidal layer of the hippocampus (arrows). Numerous nerve cells of the dentate gyrus (DG) and of its hilum have disappeared. No change is seen in the fimbria (Fi). Cresyl violet stain. D. Topography of the gliosis in the hippocampus (HIP) and fimbria (Fi). DG, dentate gyrus. Holzer stain. E. Patchy demyelination of the alveus (A), and severe demyelination of the remainder of the hippocampal formation. Spielmeyer's myelin sheath stain.

In the frontal region the architecture was fairly well preserved although at the cortico-medullary junction, and occasionally in layers V and VI, rather marked, diffuse and perivascular infiltrates could be seen. These infiltrates were composed mainly of plasma cells. In certain areas, especially at the crests of the gyri, there was a slight subpial gliosis.

Throughout the white matter, especially at the level of the corpus callosum, there were heavy perivascular infiltrates in which numerous Mott cells were found. These cells were both within the adventitia and in the nearby brain substance. The infiltrates were even heavier in the subependymal region, especially over the roof of the ventricles. In addition there was a diffuse gliosis of the white matter (Fig. 2A). Lesions of this kind were also found in the calcarine region of the occipital lobe, and the subependymal infiltrates seemed even more severe in that region.

One block studied included the temporal lobe, the fusiform gyrus, and the hippocampal gyrus. There was diffuse gliosis of the white matter (Fig. 2B). The temporal gyri showed cellular infiltration into the molecular layer, while in the fusiform gyrus there was widespread infiltration. The hippocampal formation was severely damaged. The pyramidal layer had lost almost all of its cells and was spongy; Sommer's sector as well as adjacent areas were involved (Fig. 2C). The neurones of the fascia dentata were much reduced in number and those remaining were shrunken and hyperchromatic. The cells of the hilus of the hippocampus were intact. A dense gliosis was found in all areas where there was cellular rarefaction (Figs. 2B and 2D). Demyelination in these areas was also pronounced (Fig. 2E).

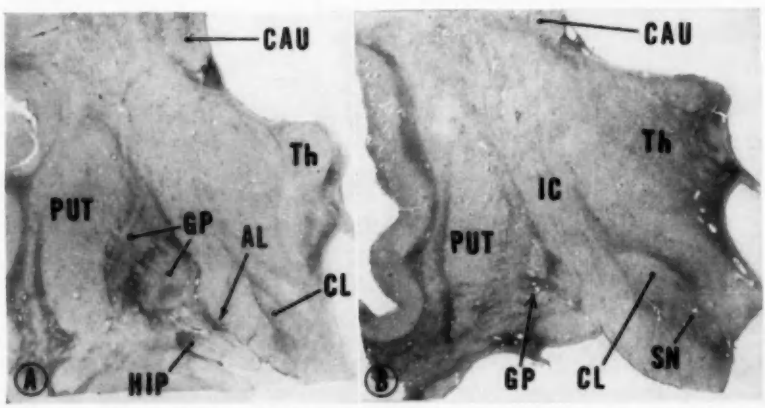

FIG. 3. A. Gliosis of medial thalamic nuclei (Th), capsule of corpus Luysi (CL), globus pallidus (especially its lateral segment and its medullary laminae) (GP), and the ansa lenticularis (AL). There is gliosis also of the white matter lateral to the putamen (PUT), beneath the caudate nucleus (CAU) and in the hippocampal formation (HIP). B. Section slightly caudal to that illustrated in A. There is gliosis of medial thalamic nuclei (Th), globus pallidus (GP), capsule of corpus Luysi (CL), substantia nigra (SN), and of the subcortical and deeper white matter. A. and B., Holzer stain.

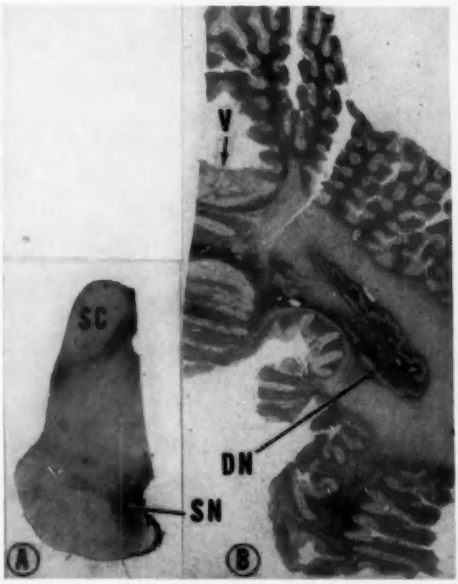

FIG. 4. A. Upper midbrain. There is peri-ependymal gliosis of the medial third of the substantia nigra (SN) and ventral to the superior colliculus (SC). B. Cerebellum, showing advanced gliosis within and bordering the dentate nucleus. The gliosis continues medially into the vermis (V). There is also rather prominent gliosis of the white matter of individual folia. A. and B., Holzer stain.

Although the different thalamic nuclei did not show gross reduction in the number of nerve cells, there was gliosis of all the lateral nuclei, and fairly severe nerve cell loss in the thalamic nuclei adjacent to the third ventricle, especially in those below the massa intermedia (Figs. 3A and 3B). Numerous infiltrations were seen in fields H_1 and H_2 of Forel as well as in the corpus Luysi and environs. In the rostral part of the substantia nigra there was conspicuous cell damage accompanied by gliosis (Figs. 3B and 4A). The lateral segment of the globus pallidus seemed even more severely involved, with a marked reduction of nerve cells, while the putamen and the caudate nuclei showed no obvious change (Figs. 3A and 3B). In the lateral geniculate body there were a few areas of infiltration and nerve cell loss.

A section passing through the upper midbrain revealed that the lateral and middle parts of the substantia nigra were intact and that the central white matter of the tegmentum contained a few perivascular infiltrates. The extraocular motor nuclei appeared normal.

As to the cerebellum, the roof nuclei on both sides had lost some cells, as had also the dentate nucleus. These cellular losses were focal, suggesting that they were secondary to an inflammatory process. An intense perivascular reaction with edema was found in the hilus of each dentate nucleus. The central white matter

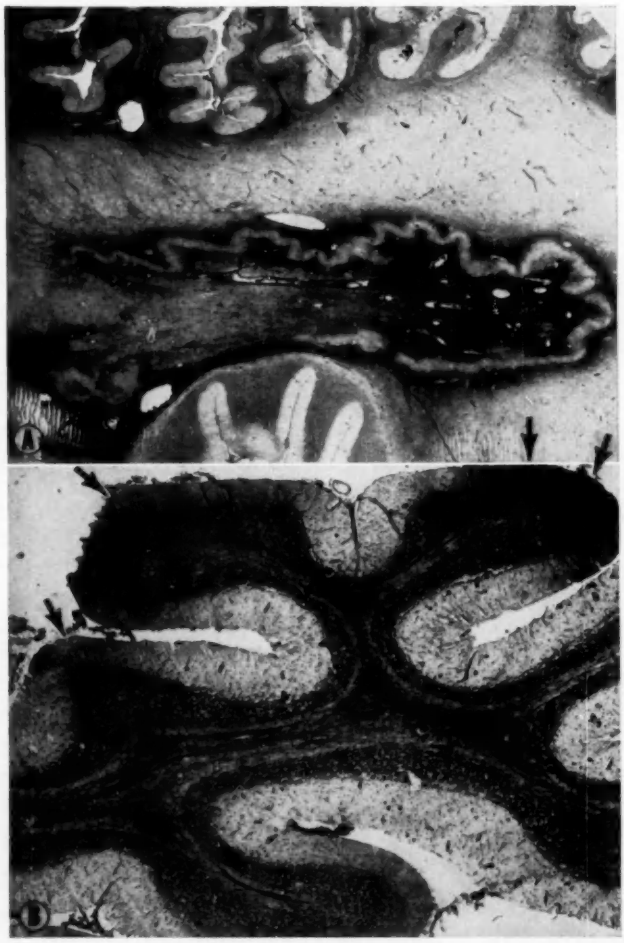

FIG. 5. A. Enlargement of a field in Fig. 4B., showing in more detail the features just described. B. Greater detail of gliosis of the white matter of folia. The glial proliferation is greatest at nodal points of the white matter. Some parts of the molecular layer are gliotic and atrophied (arrows). Holzer stain.

of the cerebellum showed a rather marked gliosis, especially in the vermis (Figs. 4B, 5A and 5B). There was a fairly diffuse infiltration which was predominantly pericapillary. These infiltrates increased in severity as the periphery was approached, being largest in the intrafolial white matter. Here, star-shaped gliotic collections and small perivascular cuffs were frequently encountered: in some places there was focal gliosis alone, and in others, gliosis with infiltrates. The process also involved the cerebellar cortex. In the most severely involved areas

all the Purkinje cells had disappeared and in their place were many glial networks; also the granular layer was rarefied, with only the Golgi cells remaining. Less severe lesions were characterized by a normal layer, infiltrates only in the subpial region of the molecular layer, loss of a few Purkinje cells, and early dissociation of the layer of Lannois-Paviot. In the most severely affected areas of the cerebellar folia, it seemed that the axial infiltration and the meningeal reaction were directly proportional to the parenchymal damage.

In short, no part of the cerebellum was spared by the pathological process and it was unusual to find a folium with more than a quarter of its usual number of Purkinje cells. The lesions were as severe in the vermis as in the hemispheres.

Changes in the medulla oblongata were rather inconspicuous. There were a few perivascular cuffs and a few glial nodules in the floor of the fourth ventricle. The restiform bodies and the pyramids were intact. In the inferior olivary nuclei some of the nerve cell were very small and in occasional areas there was perivascular nerve cell rarefaction. Also the inferior olivary nuclei were the seat of gliosis (Fig. 6A).

Two levels of the spinal cord were examined (Figs. 6B and 6C). The first, the uppermost cervical, showed a glial-cellular infiltration of the posterior column and of the crossed and uncrossed pyramidal fibers with only a mild loss of cells in the anterior horns, the remaining motor cells appearing normal. The second

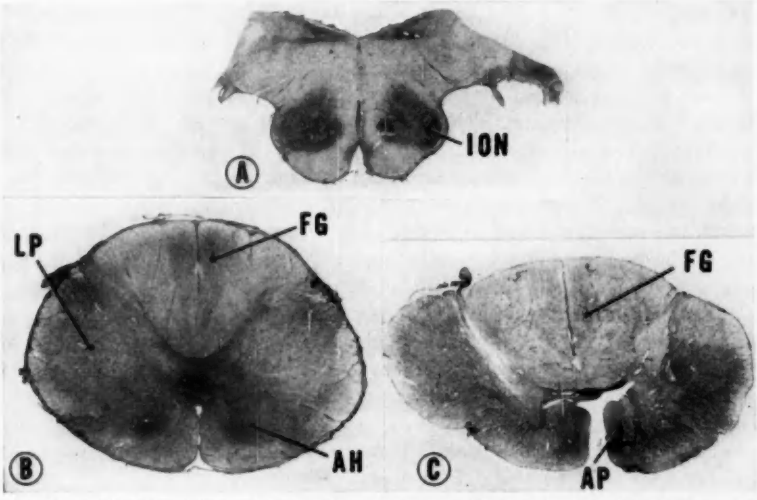

FIG. 6. A. Medulla oblongata. There is conspicuous gliosis of the inferior olivary nucleus (ION). The degree of staining in the floor of the fourth ventricle (i.e., the gliosis) is somewhat more pronounced than normally. The dark-stained (gliotic) extension to the right is cerebellar white matter. B. Uppermost cervical cord. There is prominent gliosis of the fasciculus gracilis (FG) and the anterior horns (AH), and slight gliosis of the lateral pyramidal tract (LP). C. Spinal segment C 4. Gliosis of the fasciculus gracilis is less intense than in B. The anterior pyramidal tract (AP) also shows fibrillary gliosis. Holzer stain.

section was from segment C 4: it revealed a more severe nerve cell loss, with many of the surviving cells being atrophied or otherwise distorted; in addition there was marked gliosis of the fasciculus gracilis and slight gliosis of the fasciculus cuneatus. The anterior columns also showed a definite gliotic reaction.

Examination of myelin stained sections revealed widespread changes. There was diffuse pallor of the white matter of the centrum ovale, more marked near the ventricles and the caudate nucleus than peripherally. Subcortical fibers were almost completely spared. The hippocampal gyrus was better preserved than the adjoining fusiform and temporal gyri. In the central gray matter, the thalamus appeared normal. There was marked demyelination of the globus pallidus with considerable diminution of the myelin of the strio-pallidal fibers. The myelin loss was most marked in the caudal part of the globus pallidus, though it was present also anteriorly. The striato-pallidal fibers in the putamen were also affected. Demyelination had also occurred in fields H_1 and H_2 of Forel. In the cerebellum, there was demyelination of the centrum ovale with relative sparing of the axial fibers of the folia, but the fibers of the hilum of the dentate nucleus and those immediately surrounding the dentate nucleus were demyelinated. The cerebellar peduncles and the pons were little involved except for slight pallor of the pyramidal tracts. The medulla oblongata showed little myelin, the loss being most severe in the pyramidal tracts. These tracts, both crossed and uncrossed, were rather severely involved in the cervical cord where the fasciculus cuneatus and all but a thin sector of the fasciculus gracilis (midway between the dorsal septum and the fasciculus cuneatus), were equally involved in the demyelination process.

Frozen sections stained by the Holzer method were prepared from all levels described in the foregoing. The anisomorphic character of the gliosis affecting the white matter as well as its severity were most striking. It extended all the way out to the cortex (Figs. 2A and 2B); it involved all lobes from the frontal to the occipital region, being most marked around the ventricles, especially around the posterior horns. A severe subependymal gliosis was found at the level of the thalamus, involving the posterior white commissure and being especially severe and widespread in the area of the thalamic nuclei around the third ventricle (Figs. 3A and 3B). The corpus Luysi, particularly its capsule, and the apex of the globus pallidus shared the heavy gliosis (Figs. 3A and 3B). On the whole, the globus pallidus showed a uniform gliosis, most marked in the medullary laminae. There was only minimal involvement of the ansa lenticularis (Fig. 3A).

As to the cerebellum, its dorsal white matter showed rather marked gliosis with extension into the folia (Figs. 4B, 5A and 5B). Frequently the gliosis was continuous with gliotic areas in the molecular layer. There was also marked gliosis of the hilum and of the perinuclear fibers of the dentate nucleus as well as of the vermis. In the medulla oblongata, gliosis was noted in the periependymal area and within, as well as around the inferior olivary nucleus (Fig. 6A). The nuclei of the floor of the fourth ventricle were also involved by the gliotic process. The pons was comparatively intact. The gliosis was found involving the anterior

horns of the cervical spinal cord, the lateral and anterior pyramidal tracts and the fasciculus gracilis (Figs. 6B and 5C). The gliosis of the pyramidal tracts and of the fasciculus gracilis seemed to become more severe caudalward, but this impression could not be substantiated because no sections from the thoracic and lumbosacral segments were available for comparison.

In summary, there was diffuse inflammatory infiltration of the periphery of the white matter and in the periventricular areas, accompanied by an anisomorphic fibrillary gliosis. Cortical lesions were few. The globus pallidus and corpus Luysi were severely involved, the cell loss being striking. There were disseminated lesions in the cerebellar cortex with loss of Purkinje cells, gliosis of the fibe systems of the dentate nucleus and sclerosis of the dentate and inferior olivary nuclei. In addition, in the spinal cord, there was moderate cell loss in the anterior horns of the cervical region with gliosis of the pyramidal tracts and of the fasciculus gracilis.

DISCUSSION

The clinical picture is rather easily explained by the brain alterations revealed by histologic study: the involvement of the basal ganglia, of the cerebellum and of the long tracts accounts for the combination of choreoathetosis, a tabetic-cerebellar ataxia, and pyramidal tract signs.

It would be hazardous to state that the mental deficiency can be explained on the basis of the diffuse involvement of the cerebral white matter. In spite of the belief expressed by Gallais of Marseilles and his coworkers (1939, 1952) that the endocrine changes can be explained by hypothalamico-hypophysial lesions, it would be difficult to express such an opinion in the present case.

We have attempted to interpret the significance of the findings in this case within the framework of the known clinical manifestations of trypanosomiasis as reported in the literature:

Rodhain (1943) stated that gait and equilibratory disturbances are rather frequent. He also pointed out that the gait is more "drunken" than purely cerebellar. The neurological examination of the case reported by Borremans and van Bogaert (1933) revealed a drunken gait and a combined basal ganglia-cerebellar type of dysarthria. Subsequent examination of the same patient once the disease had progressed revealed severe incoordination of the upper limbs with marked intention tremor. Sicé (1937) believed that in these patients the presence of titubation, dysmetria, hypermetria, adiodochokinesia and motor incoordination were manifestations of a cerebellar syndrome, but Gallais (1939) said that, as far as he knew, no case presenting a pure cerebellar syndrome had ever been reported, although he admitted that cerebellar signs and symptoms are particularly frequent during the course of sleeping sickness. He also added that the "drunken gait," so often seen, suggests cerebellar involvement. Actually, he felt that true cerebellar signs were not found in that he had never observed really convincing demonstrations of dysmetria, past-pointing, adiadochokinesia, etc. He believed that these pseudocerebellar signs were actually manifestations of thalamo-hypothalamic disturbances.

Chalgren and Baker (1947) wrote that cerebellar signs were frequently found and were most striking, especially during the period when the encephalitic phase of the disease became more apparent. On the other hand, Gallais and Badier (1952) wrote that the stato-kinetic difficulties attributed to cerebellar dysfunction by most authors, seemed to be, after careful observation, the result of an obvious or of an incomplete "sleepy" state.

The neuropathological literature, on the other hand, contains many reports of cerebellar lesions. As long ago as 1910, Martin and Darré observed diffuse lesions in trypanosomiasis and noted that the cellular and fiber lesions were reminiscent of those of general paresis except that they were more marked in the cerebellum. In 1933, Borremans and van Bogaert made the following comment in discussing their case:

> "In the cerebellum, the lesions involve both the intrafolial matter and the efferent systems of the dentate nuclei. In certain folia the Purkinje cells are either destroyed completely or are damaged more or less severely. Many newly formed blood vessels, many rod cells and, in the subpial areas, plasmacytic infiltrations, can be found in the molecular layer. The granular layer retains its normal density. The dentate nuclei have a rather striking thinned-out appearance: the ganglion cells are atrophied or shrunken and their inner structure is hidden by an abnormal amount of lipochrome, which stains an opalescent green."

Experimental work by van Bogaert and DeWulf (1938) has confirmed this "cerebellar fragility." In the monkey (*Papio jubilaeus*) experimentally injected with *T. gambiense* they noted marked parenchymatous alterations of the dentate nucleus, and much less severe alteration of the white matter. They found that the inflammatory process seemed to show a predilection for the cerebral cortical grey matter, especially anteriorly, for the thalamic and hypothalamic nuclei, and for the dentate nuclei. Van Bogaert (1939) working with *T. marocanum* infection in the dog, found that the cerebellar involvement was predominant in the folia facing the choroid plexus of the ventricular roof and that it consisted of an intense infiltrative process with secondary parenchymatous lesions; the Purkinje cells and the dentate neurones frequently were found to show a homogenized degeneration or coagulation.

Urtubey and Alcober (1934) made much of the regional changes in microglia of the human brain, but also noted that in the cerebellar cortex the proliferated microglia were mostly of the large branched form, especially near ganglion cells and blood vessels. Bertrand *et al.* (1935) pointed out that in the cerebellum, the Purkinje cells were disappearing, that the molecular layer contained many rod cells, and that many of the cells of the dentate nucleus were atrophied and contained abnormal amounts of lipochrome. The granular layer retained its normal density. Thus, the cerebellum contained severe parenchymatous lesions which seemed to be the result of the severe inflammatory lesions present in the meninges. The Purkinje cells were swollen or atrophied and a few had disappeared. Some had double nuclei. The fine granularity of their dendrites contrasted with the integrity of the climbing fibers and the normal appearing basket arrangement. The star-cells of the molecular layer frequently appeared spongy or vacuolated.

Fattovich and Lenti (1940) described in the intrafolial cerebellar white matter, many vacuolated areas with numerous morula-like bodies in all layers. The Purkinje cells were found to have a granular cytoplasm and irregularly thickened arborizations. The neuroglia were hypertrophied and increased in number. Finally, Gallais and Badier (1952) pointed out that the cerebellar lesions are of the same type as those found in the cerebral hemispheres but that they are less severe in the cerebellar white matter. They noted neuron hyperchromia and loss in the dentate nuclei and the presence of a "glial capsule" around the dentate nuclei. The cell loss was most striking in the medial ventral and dorsal portions of the nuclei. The Purkinje cells often appeared entirely normal and the granular layer was intact except for an increase in Bergmann's neuroglia. In view of all these reports, it is evident that the cerebellum is not only involved in encephalitic trypanosomiasis, but that the involvement is rather severe.

SUMMARY AND CONCLUSIONS

A young colored patient with a syndrome of ataxia and chorea simulating a spastic hereditary ataxia was found to have the classical cerebello-extrapyramidal form of encephalitic trypanosomiasis. The encephalitis was in the form of a systemic involvement usually associated with the true abiotrophies, namely, dentato-cerebellar, olivo-cerebellar, pyramidal, posterior column and luysi-pallidal degeneration. The adiposogenital syndrome with cryptorchidism seen in this patient can be found in cases of true abiotrophy. Since the testes and other endocrine glands were not available for examination, it is impossible to ascribe the endocrine disturbances to a central autonomic lesion. However, judging from the hypothalamic lesions present, it is conceivable that they may have had a role in the production of the endocrinopathy. The present case provides one more example for those investigators who insist that the genital system is highly vulnerable in sleeping sickness. It is of great theoretical interest to find that an inflammatory disease such as trypanosomiasis may, like syphilis, cause degeneration of fiber systems in such a manner that the clinical appearance becomes that of an heredodegenerative disease.

REFERENCES

Bertrand, I., Bablet, J., and Sicé, A., 1935. Lésions histologiques des centres nerveux dans la trypanosomiase humaine (à propos de deux cas mortels non traites), *Ann. Inst. Pasteur* **54:** 91–147.

Borremans, P., and van Bogaert, L., 1933. Les manifestations extrapyramidales de la trypanosomiase chez l'Européen (syndrome d'inhibition avec stéréotypies, pigmentation cutanées symétriques et anneau cornéen), *J. Belge Neurol. et Psychiat.* **33:** 511–588.

Chalgren, W. S., and Baker, A. B., 1947. The nervous system in tropical diseases—a clinical review, *Medicine* **26:** 395–439.

Fattovich, G., and Lenti, P., 1940. Ulteriore contributo all'istopatologia dei ,centri nervosi nella tripanosomiasi umana africana, *Ann. Patol. Trop. e Parasit.* **1:** 219–255.

Gallais, P., 1939. Les formes neuropsychiques de la trypanosomiase humaine africaine. (Essai d'une systématisation clinique et physiopathologique). In *Les Grandes Endémies Tropicales*, Vigot Frères, Paris, pp. 113–151.

Gallais, P., and Badier, M., 1952. Recherches sur l'encéphalite de la trypanosomiase humaine africaine. Corrélations cliniques, anatomiques, électroencéphalographiques, biologiques, *Méd. Trop.* **6:** 633–675.

MARTIN, L., AND DARRÉ, H., 1910, cited by Borremans and van Bogaert (1933).
RODHAIN, J., 1943. Maladie du sommeil. Clinique et diagnostic, *Arch. Méd. Belges* **96**: 1–12.
SICÉ, A., 1937. *La Trypanosomiase Humaine en Afrique Intertropicale*, Vigot Frères, Paris.
URTUBEY, L., AND ALCOBER, V., 1934. Contribución al estudo histo-patologico de los centros-nerviosos en la enfermedad del sueño, *Rev. Espan. Biol.* **3**: 177–219.
VAN BOGAERT, L., 1939. Etude sur le mode d'extension et l'histopathologie des trypanosomiases expérimentales. II. La méningo-encéphalite à trypanosoma marocanum chez le chien, *J. Belge Neurol. et Psychiat.* **39**: 296–319.
VAN BOGAERT, L., AND DEWULF, A., 1938. Etudes sur le mode d'extension et l'histopathologie des trypanosomiases expérimentales, *J. Belge Neurol. et Psychiat.* **39**: 559–582.

CONSERVATION OF *TRICHOMONAS* IN MONOBACTERIAL CULTURES

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Until methods of preserving *Trichomonas* at low temperature have been definitely developed (McEntegart, 1954), a great obstacle to conservation of cultures of these parasitic protozoa is the need of performing transfers at very brief intervals. In media of the CPLM type (Johnson and Trussell, 1943), which permit the growth of *Trichomonas* only in the absence of bacteria (Williams and Plastridge, 1946), *Trichomonas vaginalis* and *T. foetus* must be transferred every 48 hours at 37°C. Behaving analogously is a strain of *T. hominis* recently freed from bacteria (de Carneri, 1955).

In general, the vitality of these flagellates is preserved much longer in media that permit their growth in association with other micro-organisms. Sorel (1954) affirms that *T. vaginalis* grows better in the presence of fungi of the *Candida* genus. The conservation of a local strain of *T. vaginalis* of our collection in the presence of *Candida albicans* ATCC 2091 in CPLM medium, requires a transfer every 7 days.

Lamy (1953), using a diphasic medium of horse serum with rice powder, cultivates *Trichomonas hominis* at 25°C. with mixed bacterial flora by transferring it every 8 days, and *T. vaginalis* at 37°C. with mixed flora with transfers every 6 days. In this medium the protozoa do not grow in the absence of bacteria (McEntegart, 1954; de Carneri, 1955).

The advantages of the conservation of *Trichomonas* in the presence of bacteria are considerable, due both to lower cost of the media and to lesser frequency of transfer. When this form of conservation is adopted, however, a rapid method of isolation is necessary, starting from contaminated cultures, to obtain pure cultures of flagellates for the laboratory uses that require them.

The problem, which is anything but simple (McEntegart, 1954), can nevertheless be simplified when *Trichomonas* is cultivated in the presence of a single, appropriately chosen bacterium. A method is described here for transferring *Trichomonas* from polybacterial cultures to pure cultures, and *T. hominis*, *T. vaginalis* and *T. foetus* from pure cultures to cultures of these protozoa in the presence of a strain of *Escherichia coli*.

EXPERIMENTAL

Strains of Trichomonas. Recently-isolated local strains of *T. vaginalis* and *T. foetus* were used, and a strain of *T. hominis* associated with mixed bacterial flora, kindly sent to me by Prof. Lamy of the Pasteur Institute of Paris. *Diphasic horse-serum medium.* Two cc. of sterile horse serum are coagulated in inclined bacteriological test tubes by heating for 20 minutes at 80°C., in a suitably equipped water-bath. A spatula of rice starch powder is added, and 5 cc. of a

mixture of 15 parts sterile horse serum and 85 parts Ringer's solution autoclaved without $NaHCO_3$ and with the addition of 100 μg per cc. of sodium bicarbonate sterilized by filtration through sintered glass filter. This medium can be kept for months at +5°C.

CPLM medium. The medium used is a modification of the CPLM medium of Johnson and Trussell (1943). Its composition is: Bacto Peptone, 32 gm.; Bacto Agar, 1.6 gm.; Maltose, 1.6 gm.; NaCl, 5.76 gm.; $NaHCO_3$, 0.096 gm.; $CaCl_2$, 0.096 gm.; KCl, 0.096 gm.; H_2O, 960 cc. Add 24 gm. of Bacto Liver suspended in 320 cc. water, heated for 1 hour at 65°C., precipitated by boiling for 5 minutes and filtered. When the agar and the other ingredients have dissolved after heating, 2.1 gm. of L-cysteine and 0.7 cc. of methylene blue 0.5% are added. The pH is adjusted to 6. The medium is distributed in volumes of 9 cc. into bacteriological test tubes and sterilized for 30 minutes at 112°C. One cc. of human serum is added sterilely to each tube. The medium is kept at 25°C. to avoid precipitation of the agar, and can be used for 2 weeks at most, owing to progressive oxidation of the cysteine.

Isolation of Trichomonas *from contaminating micro-organisms.* The contaminated strains are transferred into CPLM medium to which have been instantly added 1000 μg/cc. of dihydrostreptomycin and 1000 I.U./cc. sodium penicillin. Chloramphenicol may also be used, at non-protozoostatic concentrations, lower than 50 μg/cc.

When fungi or other non-motile contaminants are present, it is useful to seed the contaminated material into an arm of a "W" tube filled with CPLM medium with the above antibiotics added and, after 2 days of incubation at 37°C., to isolate the *Trichomonas* that are displaced towards the other arm of the tube. The method has been described for *T. vaginalis* (de Carneri, 1955) but gives identical results with *T. hominis* and *T. foetus*, which possess (especially the latter) much greater motility.

After two transfers in the presence of the above quantities of penicillin and streptomycin, the *Trichomonas* cultures are usually pure, as demonstrated by tests for sterility in Bacto Fluid Thioglycollate medium and Sabouraud's bouillon.

The *Trichomonas* strains can then be cultivated at 37°C. in CPLM medium without antibiotics. The speed of growth decreases, in the order of *T. foetus* to *T. vaginalis* to *T. hominis*, and for conservation of the strains by means of daily transfers it is expedient to transfer 0.1, 0.15, and 0.2 cc. respectively from the 24-hour cultures into 10 cc of the fresh cultures. In this way *Trichomonas* can reach concentrations of between 1,000,000 and 2,500,000/cc. Cultures thus concentrated quickly lose their vitality through accumulation of toxic products in the medium. As a rule no protozoon survives after 3 to 5 days.

Cultures of Trichomonas *with* Escherichia coli. Test tubes containing the diphasic horse-serum medium described above are seeded with a loopful of a culture of *Escherichia coli* grown on meat agar. I have used the *E. coli* 120 strain of our collection. One drop of the pure cultures of *Trichomonas* in CPLM medium without antibiotics is added to the test tubes (ca. 100,000 protozoa) and the new cultures incubated at 37°C. for *T. foetus* and *T. vaginalis*, and at 25°C. for *T.*

hominis. The first two species do not grow at this temperature, nor do they keep long when, after an initial growth at 37°C., they are placed at 25°C.

In these and in successive cultures in diphasic medium the three species of *Trichomonas* do not exceed concentrations of 200,000 per cc., reached in less than a week. *T. foetus* and *T. vaginalis* remain viable more than a month, *T. hominis* more than 4 months (*see Table 1*). *Escherichia coli* 120 reaches concentrations of 300,000,000 per cc.

Evaporation through the cotton plugs progressively reduces the volume of the liquid in the test tubes, and the death of the protozoa under these conditions occurs practically when the last tenths of cc. of the liquid have been reduced to a thick paste. Preliminary tests carried out on *T. hominis* cultivated in hermetically-plugged test tubes have not, however, prolonged the effective period of conservation.

For conserving the strains I have adopted a system of fortnightly transfers at 37°C. for *T. foetus* and *T. vaginalis*, and bimonthly transfers at 25°C. for *T. hominis*, which has proved to be well within safe limits. The volume of the "seed" is between 0.1 and 0.3 cc.

Freeing Trichomonas *from* Escherichia coli. Preparation of bacteria-free cultures of *Trichomonas* in CPLM medium, starting from cultures contaminated by *E. coli*, is very simple, owing to the bactericidal action of dihydrostreptomycin. Table 2 shows the bactericidal action of 1000 μg/cc. dihydrostreptomycin on *E. coli* 120 suspended at a concentration of 200,000,000/cc. in CPLM medium.

TABLE 1

Maximum time of survival of Trichomonas *associated with* Escherichia coli 120 *in diphasic horse-serum medium*

Species	*Trichomonas hominis*	*Trichomonas foetus*	*Trichomonas vaginalis*
Incubation temperature	25°C	37°C	37°C
Days	126	38	39

TABLE 2

Bactericidal activity of 1000 μg/cc. dihydrostreptomycin against *Escherichia coli* 120 suspended at a concentration of 200,000,000/cc. in CPLM medium at 37°C. At time intervals, 0.001 cc., initially containing 200,000 bacteria and 1 μg dihydrostreptomycin, was taken and seeded in 25 cc. of Bacto Fluid Thioglycollate medium. Control of these tests for sterility was carried out after 4 days of incubation at 37°C.

Times of withdrawal	Results
0 min.	+
15 min.	+
30 min.	+
1 hour	+
1 hr. 30 min.	+
2 hours	−
3 hours	−

After 2 hours at 37°C. no bacterium survived. Analogous results are obtained if penicillin is present in addition to dihydrostreptomycin. In practice, 0.1 cc. of a mature culture of *T. hominis*, *T. foetus* or *T. vaginalis* in diphasic medium is transferred to a test tube containing 10 cc. of CPLM medium rendered bactericidal by the addition of 1000 μg/cc dihydrostreptomycin and 1000 U/cc penicillin. Pure cultures of *T. hominis*, *T. foetus* or *T. vaginalis* are obtained by incubation at 37°C., as may be demonstrated by microscopic examination and normal sterility tests in Bacto Fluid Thioglycollate medium.

SUMMARY

Trichomonas foetus, *Trichomonas hominis* and *Trichomonas vaginalis* live much longer in media permitting their growth in the presence of bacteria, than they do in pure cultures in CPLM medium. In a diphasic medium of horse serum and rice starch, *T. hominis*, associated with a pure strain of *E. coli* and incubated at 25°C., keeps for more than 4 months; *T. foetus* and *T. vaginalis*, incubated at 37°C. with *E. coli* will keep for more than a month. Comparing these periods of time with those cited by Lamy (1953), who keeps *T. vaginalis* and *T. hominis* in the presence of mixed bacterial flora, it may be observed that better results are obtained by freeing the flagellates from the mixed flora and associating them with a pure strain of *E. coli*. To transfer from cultures mixed with bacteria to pure cultures in CPLM medium, it is sufficient to make one transfer in this medium with the addition of 1000 μg/cc. dihydrostreptomycin and 1000 U/cc penicillin.

The method of conserving strains of *T. hominis*, *T. foetus* and *T. vaginalis* here described requires fewer transfers than the methods hitherto in use.

REFERENCES

DE CARNERI, I., 1955. *Trichomonas hominis* cultured without bacteria, *Nature*, **176**: 605.

DE CARNERI, I., 1956. Isolation of *Trichomonas vaginalis* from fungi and bacteria, *Am. J. Trop. Med. Hyg.* **5**: 210–212.

JOHNSON, G., AND TRUSSELL, R. E., 1943. Experimental basis for the chemotherapy of *Trichomonas vaginalis* infestations. I. *Proc. Soc. Exp. Biol. Med.* **54**: 245–249.

LAMY, L., 1953. Technique générale de culture des protozoaires parasites des voies digestives et génitales, *Extrait de la Biologie Médicale* **42**: 1–64.

MCENTEGART, M. G., 1954. The maintenance of stock strains of trichomonads by freezing, *J. Hyg.* **52**: 545–550.

SOREL, C., 1954. Trois téchniques de recherche du "*Trichomonas vaginalis*"; leurs valeurs comparées, *La Presse Méd.* **62**: 602–604.

WILLIAMS, L. F., AND PLASTRIDGE, W. N., 1946. Use of antibiotic substances for freeing *Trichomonas foetus* from bacteria, *J. Bact.* **51**: 127.

HYDROXYCHLOROQUINE IN THE TREATMENT OF MALARIA

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Since the introduction in 1924 of the first synthetic drugs for the treatment of malaria, several chemical series have been used for this purpose. The effectiveness of an antimalarial drug should be considered not only from the clinical, but also from the public health viewpoint. Drugs which are not restricted to use by the physician, his assistants or even patients of higher educational standards, are the most indicated, since malaria is a disease of the masses that mainly strikes the rural population—generally the less educated in every country—and in some areas of the world even the most primitive people. Under these conditions, the drug of choice must have low toxicity and high antimalarial potency in order to permit a single dose or at least a one-day treatment. Experiences in Latin America and India with quinine and quinacrine show that patients generally discontinue the treatment as soon as the main symptoms disappear.

Though the 4-aminoquinolines do not meet all the requirements of the perfect antimalarial, largely because of their ineffectiveness in the complete eradication of *P. vivax*, they are the most useful for medical practice (Nieto-Caicedo 1948, 1951). The reasons for their usefulness are: high antimalarial potency against every species of *Plasmodium* without producing resistant strains; low toxicity which permits short treatments and even single doses in semi-immune populations; high tissue fixation and slow rate of excretion and degradation, maintaining persistent blood levels with small weekly suppressive doses. On the other hand, the lack of gametocide action of the 4-aminoquinolines against *P. falciparum* is not a serious short-coming because they rapidly control parasitemia (when used for mass treatment) producing indirect gametocidal effects.

Chloroquine acts as an atoxic or low-toxic drug in clinical tests, although it occasionally produces signs of intolerance. It was studied by Nieto-Caicedo (1951) for clinical and chemotherapeutic control of malaria in certain areas of Venezuela where morbidity and transmission could not be controlled with DDT alone. During the last 10 years millions of tablets of chloroquine have been distributed to rural populations without any serious accidents even though many errors in dosage have undoubtedly been committed.

HYDROXYCHLOROQUINE[1]

Hydroxychloroquine differs from chloroquine in that a hydroxyethyl group is substituted for one of the ethyl groups on the tertiary amino nitrogen (Brodie *et al.*, 1947, Surrey and Hammer, 1950). Acute intravenous, intraperitoneal and oral toxicity studies in mice show that hydroxychloroquine is only about one-half

[1] Plaquinol® (brand of Hydroxychloroquine) Sulfate, also known in U.S.A. as Plaquenil® Sulfate, supplied by Winthrop Products Inc., New York, N. Y.

as toxic as chloroquine by the intravenous and intraperitoneal routes and only one-fifth as toxic when administered orally.

Antimalarial activity. Experimental studies (personal communication from the Medical Research Department, Winthrop Laboratories, Inc., New York, N. Y.) with *P. lophurae*-infected ducks showed that hydroxychloroquine given daily at the optimal dose of 20 mg. per kilogram of body-weight acts faster than quinacrine and its activity is 5.5 times more effective. In comparison with chloroquine, it was equally effective in the control of parasitemia. In the prophylactic suppression of malaria an initial dose of 600 mg. of hydroxychloroquine base followed by 300 mg. of base weekly maintains an effective plasma concentration.

Field studies by Clark (1954), Hoekenga (1953, 1954, 1955), and Loughlin *et al.* (1952) confirm its suppressive action against relapsing malaria when administered once a week. According to Clark, the addition of primaquine to schizontocides for weekly suppressive medication does not improve the results. Table 1 summarizes the results of Loughlin *et al.* in 1952 and Hoekenga in 1955 using hydroxychloroquine in the treatment of malaria.

Studies in Venezuela. With the introduction of hydroxychloroquine, the following studies were carried out on 50 patients. Because of the highly effective

TABLE 1

Therapeutic action of hydroxychloroquine

Dosage	No. patients	Type of malaria	Parasites disappeared		Temperature became normal
			Hours	Additive %	
					hrs
Loughlin *et al.*, 1952					
Single oral dose			12	12	
Adults: 1,200 mg. base			24	46.6	
			48	78.6	
Children 6–12: 900 mg. base	75	P. falciparum	72	90	
			96	96	*
Under 6: 600 mg. base					
Hoekenga, 1955					
Single oral dose					
450 mg. base	14	P. falciparum	38.2		26.2
	11	P. vivax	33.5		28.3
Single oral dose					
750 mg. base	50	P. falciparum	30.3		20.9
	75	P. vivax	24		18.7
Single intramuscular dose					
360 mg. base	37	P. falciparum	33		21.7
Single intravenous dose					
360 mg. base	26	P. falciparum	21.5		24.3†

* Slight signs of intolerance in a few cases. Parasites were found in one case until the 6th day. Asexual forms were present in two cases on the 8th day.

† No toxic effects were noted on any of the regimens. No adverse consequences were observed on patients with pregnancy, severe malnutrition or severe anemia.

measures taken in the malarial regions of Venezuela, there was a shortage of patients for a wider study. These studies were carried out during 1953–54 in a rural area located south of Lake Maraciabo, in the States of Trujillo and Zulia. The area is covered by jungle and the climatic conditions are similar to those of the malarial equatorial zone. The main vector is *A. darlingi*, though *A. nuneztovari* is also important in the transmission of the disease. Formerly it was a highly endemic area but frequent DDT sprayings of the homes and other control measures have reduced the number of *P. vivax* cases and completely eliminated *P. falciparum* and *P. malariae*.

The 50 patients treated for malaria were mostly farmers and their families who had lived in the area for many years. Because of the few possibilities of infection, the grade of immunity was very low or nonexistent, particularly in the newcomers. The majority were mestizos (Indian-Spanish stock) with some pure whites. The ages ranged from 2 months to adults. The drug used was hydroxychloroquine sulfate in tablets of 200 mg. equivalent to 150 mg. of hydroxychloroquine base. The dosage schedule with hydroxychloroquine was planned on the basis of the dosage used in Venezuela for chloroquine. The single dose of 1 gram of hydroxychloroquine sulfate (750 mg. hydroxychloroquine base) used for adults was similar to the dosage used by Hoekenga (1955) in Honduras, but smaller than the amounts given by Loughlin *et al.* (1952) in Haiti or Treviño Villaseñor (1953) in Mexico. The dosage schedule is given in Table 2. The drug was given under the personal supervision of the author. One, two or three daily blood smears were taken for several days until they became negative. Temperature changes, other symptoms and side effects, if any, were also recorded.

Tolerance and toxicity. The drug was well tolerated in all cases. No side effects such as diarrhea, cramps, dizziness, pains or abnormalities in the urine were observed. A few patients had slight nausea or vomiting which disappeared very quickly. No unfavorable effects were observed in five women of whom two were pregnant and three were breast-feeding.

RESULTS

The results of this study agree with the observations of other workers cited above that hydroxychloroquine in a single oral dose or in multiple oral doses in one day has great effectiveness in the treatment of *P. vivax* malarial infection. Both parasitemia and temperature in 50 patients were controlled in the great

TABLE 2

Dosage of hydrozychloroquine

Age	Dosage	Method
Under 1 year	1 tablet (150 mg. base)	Single dose
1 to 2	2 tablets (300 mg. base)	One tablet plus one eight hours later
2 to 5	3 tablets (450 mg. base)	Two tablets plus one eight hours later
6 to 10	4 tablets (600 mg. base)	Two tablets plus two eight hours later
11 to 15	4 tablets (600 mg. base)	Single dose
Adults	5 tablets (750 mg. base)	Single dose

TABLE 3

Plasmodicidal activity of hydroxychloroquine and control of temperature in P. vivax *infections*

	No. of patients *P. vivax* positive before treatment	Patients who became negative each day*				Average (days)
		1st day	2nd day	3rd day	4th day and on	
	50	18	24	8	All negative	1.8
Per cent.....		36	48	16	100	
	No. of patients with fever at the beginning of treatment	Temperature returns to normal on				
		1st day	2nd day	3rd day	4th day and on	
	28	19	8	1	All normal	1.3
Per cent.....		68.5	28.5	3.5	100	

* Disappearance of all plasmodia (trophozoites, schizonts and gametocites).

majority of cases in less than 2 days (Table 3). Slight differences from other studies in the time of control of parasitemia and temperature may well be due to local differences in the strains of *P. vivax* or to the conditions of immunity of the populations involved. Results with hydroxychloroquine are comparable to those with chloroquine previously reported (Nieto-Caicedo, 1951).

SUMMARY

Hydroxychloroquine was given orally to 50 patients with *P. vivax* infections, either in a single dose or in a one-day treatment of several doses. Both parasitemia and temperature were controlled on the average in less than 2 days, with no unfavorable effects noted. This study in Venezuela confirms observations by others that hydroxychloroquine exhibits antimalarial activity against *P. vivax* comparable to that of other 4-aminoquinolines but appears to be better tolerated.

REFERENCES

Brodie, B. B., Udenfriend, S., Dill, W., and Chenkin, T., 1947. The estimation of basic organic compounds in biological material. III. Estimation by conversion to fluorescent compounds, *J. Biol. Chem.* **168**: 319.

Clark, H. C., 1954. The suppressive treatment of malaria in a rural village with primaquine and Plaquenil, *Am. J. Trop. Med. & Hyg.* **3**: 250–253.

Hoekenga, M. T., 1954. The treatment of acute malaria with single oral doses of amodiaquin, chloroquine, hydroxychloroquine and pyrimethamine, *Am. J. Trop. Med. & Hyg.* **3**: 833–838.

Hoekenga, M. T., 1953. Treatment of malaria with oral or intravenous Plaquenil, *Am. J. Trop. Med. & Hyg.* **2**: 805–807.

Hoekenga, M. T., 1955. The treatment of malaria with hydroxychloroquine, *Am. J. Trop. Med. & Hyg.* **4**: 221–223.

Loughlin, E. H., Rice, J. B., Wells, H. S., Rappaport, I., and Joseph, A. A., 1952. The treatment of *Plasmodium falciparum* malaria with a single dose antimalarial, Antibiotics & Chemotherapy **4**: 171–174.

Nieto-Caicedo, M., 1948. In Discussion of Schneider, J., Decourt, Ph., and Mechlar, D.: Nouveaux medicaments du paludisme-etude comparée de leur activité dans le traite-

ment curatis et emprophylaxie, *Proceedings of the 4th International Congress on Trop. Med. & Malaria, Washington, D. C., Department of State* **1**: 756–775.

NIETO CAICEDO, M., 1951. Experiencias venezolanas con las drogas antimalaricas de la serie 4-aminoquinolinas, *Revista de Sanidad y Asistencia Social* **16**: 317–441.

SURREY, A. R., AND HAMMER, H. F., 1950. The preparation of 7-Chloro-4-[4-(N-ethyl-N-beta-hydroxyethylamino)-1-methylbutylamino] quinoline and related compounds, *J. Am. Chem. Soc.* **72**: 1814–1815.

TREVIÑO VILLASEÑOR, A., 1953. Algunas consideraciones sobre terapéutica experimental de la malaria, *Gac. Méd. México* **83**: 469–480.

BACTERIOLOGICAL ASSAY OF SMALL BOWEL SECRETION IN TROPICAL SPRUE

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At the present time the etiology of tropical sprue is undetermined. Manson-Bahr (1948) has indicated that tropical sprue must be infective since malnutrition is not observed in many patients. Frazer (1949) has suggested that an alteration in the small bowel bacterial flora could be a mechanism for impaired absorption by competitive demands for nutrition. Thus, bacterial growth in the small bowel, which is normally bacteria-free (Cregan and Hayward 1953) could possibly lead to impaired absorption by utilization of metabolites, production of antimetabolites and/or production of toxic effects on the mucosa. Since the area of active absorption of foodstuffs is greatest in the jejunum, the present study was undertaken to sample small bowel juice in that segment for bacterial growth. It was hoped that information could be obtained to allow a more definitive conclusion regarding the role of bacteria in the etiology of malabsorption in tropical sprue.

MATERIALS AND METHODS

Patients studied with a diagnosis of sprue syndrome had a clinical history of weight loss, anorexia, lassitude and diarrhea. Not all patients were anemic. However, all untreated patients had impaired absorption by oral tolerance tests of d-xylose, butter fat and vitamin A. Those patients studied by dietary fat balance determinations had steatorrhea. All untreated patients had abnormal x-ray patterns of the small bowel after an oral barium meal. Radiologic studies were not performed in the previously treated patients. All treated patients had received oral folic acid or parenteral vitamin B_{12} therapy for at least a three month period. The volunteer patients studied had no evidence of malabsorption from their clinical history.

A clean rubber Miller-Abbott intestinal tube was prepared for intubation by removing the rubber condom covering; the proximal sampling tips were plugged with cotton and the tube autoclaved at 15 lbs. pressure for 15 minutes to allow sterile sampling. A rubber bag filled with 4 ml of mercury was attached to the distal tip of the tube with rubber cement to aid in passage through the bowel. The lower set of one lumen outlet of the tube was sealed with liquid collodion by first placing a drop or two of collodion over the surface of sterile water to form a thin film and then rolling the collodion film over the outlets. The seal was held firmly in place by applying liquid collodion around the edges of the outlets with a sterile cotton swab. The rubber bag and sealed portion of the modified Miller-Abbott tube was sterilized with merthiolate solution (1:1,000) for 15 minutes. The disinfectant was washed from the tube by thorough rinsing in sterile distilled water and wrapped in sterile brown paper until ready for use.

Before intubation, the tube was examined to determine if all outlets were sealed and if the collodion membrane allowed adequate flexing of the tube that might occur in the bowel. The tube was passed through the mouth and the progress through the stomach and duodenum observed with the fluoroscope. The intestinal tube was allowed to progress beyond the Ligament of Treitz into the jejunum for 20 cm. It was recognized that this measurement was only an approximation, for "telescoping" of the bowel over the tube suggested that the sampling was at a greater distance in the jejunum. The cotton plug from the proximal end of the sterile sealed lumen was removed and a 20 ml syringe attached. The collodion seal was broken by air pressure and jejunal juice aspirated. The samples obtained were chilled in cracked ice until they could be cultured within several hours. Satisfactory small bowel intubations were achieved in about one half of the patients studied.

The aspirated jejunal juice was inoculated on a MacConkey agar plate, two thioglycollate broth tubes and two blood agar plates, one of which was incubated aerobically and the other anaerobically. Total bacterial counts were performed by the standard agar pour plate method (Standard Methods, 1946). Various dilutions of jejunal juice were tested for bacteriostatic and/or bactericidal activity by the paper disc method (Antibiotics, 1949) against the following genera of organisms: *Micrococcus*, Diphtheroids, *Pseudomonas*, *Escherichia*, *Aerobacter*, *Alcaligenes* and *Proteus*.

RESULTS

Successful jejunal sampling was obtained in thirty-five studies. Nineteen (54 %) of the samples proved to be sterile. As may be seen in Table 1, the sterility of the cultures was distributed approximately equally between the control and sprue patients. The predominating organisms found in the positive cultures belonged to the genera *Micrococcus* and *Streptococcus*; fourteen (87 %) had one or both of these organisms in small numbers. The organisms found are listed in Table 2. Two treated sprue patients with achlorhydria had in addition gram negative bacilli; in one patient *Paracolobactrum* and *Alcaligenes*, and in the other patient a massive growth of *E. coli*. The experiments performed on the bacteriostatic and/or bacteriocidal activity of jejunum juice obtained from both sprue and non-sprue patients demonstrated no inhibitory activity against any of the test organisms. The average bacterial counts obtained were low, 9,000 per ml., with a range from 1,000 to 30,000 per ml.

TABLE 1

Number of sterile jejunum samples obtained from 35 patients

	Controls	Untreated sprues	Treated sprues
Number of jejunum samples	13	16	6
Number of sterile cultures	8	10	1
Per cent sterility	61.5%	62.5%	16.6%

TABLE 2

Types of bacteria and the number of times these organisms were isolated from the jejunum of 35 patients

Organisms isolated	13 Controls	16 New sprues	6 Old sprues
Streptococcus and/or Micrococcus	4	6	4
Alcaligenes			1
Paracolobactrum			1
E. coli			1
Diphtheroids			1
Gram positive bacilli	1	2	2
Gram negative cocci		1	1

DISCUSSION

Sampling of bowel juice at this level of the small bowel by an intubation technique has not revealed any marked increase in the bacterial population. The paucity of organisms obtained indicates that no "native flora" exists in the jejunum of sprue patients. These observations were similar to the results of Cregan and Hayward (1935) in which bowel aspirations were obtained during surgical procedures. The few micrococci and streptococci present were considered transient bacteria from the respiratory tract. Two patients with achlorhydria had a marked increase in gram negative organisms which was attributed to inadequate inhibition of bacterial growth by gastric juice.

The technique used for sampling cannot be considered entirely adequate. Despite careful handling the seal may have been broken in transit. Usually, however, adequate resistance to manual pressure on the air filled syringe suggested that the collodion membrane was intact. This area of the small bowel was chosen inasmuch as maximum absorption of foodstuffs occurs at this level. Hence, if ascending bacteria were responsible for impaired nutrition, their presence should be detected in the jejunum. The present study would not support a hypothesis of an altered bacterial flora in the small bowel at a level to interfere with absorption. A viral etiology has been postulated by Manson-Bahr (1948) but no specific observations have been reported to support such a hypothesis. The recurrence of sprue or the onset of the disease in patients who have been away from tropical areas for many years has given strong circumstantial evidence for incriminating a latent viral infection.

CONCLUSION

Studies of the bacterial population in the jejunum of patients with tropical sprue syndrome and volunteer control patients have been determined by intestinal intubation. No alteration of the bacterial flora was observed in sprue patients when compared to volunteer subjects without malabsorption. These observations suggest that the impaired nutritional absorption in the sprue syndrome cannot be attributed to the effect of an altered bacterial flora.

REFERENCES

MANSON-BAHR, P., 1948. Clinical appearance of nutritional deficiencies in tropical sprue and dysenteries, *Proc. 4th Int. Cong. Trop. Med. and Malaria*, p. 1241, U. S. Govt. Printing Office, Washington, D. C.

FRAZER, A. C., 1949. Fat metabolism and the sprue syndrome, *Brit. Med. J.* **2**: 770–773.

CREGAN, J., AND HAYWARD, N. J., 1953. The bacterial content of the healthy small intestine, *Brit. Med. J.* **1**: 1356–1359.

Standard Methods for the Examination of Water and Sewage, 1946. 9th ed., American Public Health Association, New York, N. Y.

Antibiotics, 1949. Vol. 1, Oxford University Press, London, New York, and Toronto.

AN EPIDEMIC OF HISTOPLASMOSIS IN VENEZUELA

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The results of studies of skin sensitivity to histoplasmin, made in Venezuela by Campins *et al.* (1950) and by Pollak (1953) indicated the probable existence of histoplasmosis in this country and the two proven cases of the disease, reported by Campins *et al.* and Pollak *et al.* in 1953 definitely established its existence. The purpose of this paper is to report the first epidemic of histoplasmosis recognized in Venezuela in order to contribute to a better understanding of the epidemiological aspects of this disease.

In 1943, Christie was impressed with the frequency with which pulmonary calcifications were found in persons negative to the tuberculin test. Christie (1945), Palmer (1945), Furcolow (1947) and others later showed that a high percentage of these individuals gave positive skin tests to histoplasmin. These observations led to a new appraisal of histoplasmosis as a rather common and benign infection in many areas; the disseminated and highly fatal infection which had been the only form previously recognized turned out to be relatively rare.

As for the mode of transmission, *Histoplasma capsulatum* has been found in rodents, cats and dogs (Prior and Cole, 1951) but there is no evidence that man is infected by contact with animals. Further investigations have led to the isolation of the fungus from soil (Zeiberg *et al.*, 1952; Furcolow *et al.*, 1952), from river water (Gordon *et al.*, 1952) and from the air (Ibach *et al.*, 1954). Zeiberg *et al.* (1952) concluded that the natural source of this infection would be found in the soil.

Grayston (1953) and Furcolow (1953) analyzed the findings in 13 epidemics of acute pulmonary disease of undefined etiology, registered at different times and places in the United States. All had occurred under circumstances which presented a certain similarity: the infected individuals had been cleaning chicken or pigeon houses, or, in one case, an abandoned school building; some soldiers fell sick after lodging for 6 hours in an abandoned shed; one outbreak occurred in a group which had explored a cave, as was the case in the epidemic about to be described. It was shown by serologic tests and x-rays of the 116 individuals involved in the outbreaks, together with cultivation of *H. capsulatum* from samples of the soils where the infection was thought to have been contracted, that the epidemics were almost certainly histoplasmosis.

Loosli (1954) has recently isolated *H. capsulatum* from the floor of an abandoned silo and recovered the fungus from the sputum and bone marrow of one of the 6 members of a family who contracted histoplasmosis while cleaning the silo.

The results of these etiologic studies provided sufficient data to conclude that the causal agent had been *Histoplasma capsulatum*, that the infection occurs upon inhaling the spores suspended in the air, and that its saprophytic state in nature actually constitutes the reservoir of the fungus.

THE VENEZUELAN EPIDEMIC

Two students, G. R. and E. A., aged 18 and 14 respectively, residents of Barquisimeto, Venezuela, became seriously ill in February, 1954, with a disease that lasted one month and was characterized by fever, cough (dry at first, then with slight expectoration), and thoracic pains. The tentative diagnosis during the first 2 or 3 weeks was a fever of the typhoid group, and the patients were given chloromycetin, streptomycin and penicillin, but without appreciable effect. Direct examination and cultures of sputum were negative for the tubercle bacillus and for fungi; blood examination failed to find malaria parasites.

Chest x-rays taken in March showed numerous dense nodular infiltrates disseminated over both lungs, and marked bilateral hilar filling, which in the course of two weeks became accentuated (Fig. 1). The absence of tubercle bacilli in several samples of sputum and gastric contents, the radiographic aspect of the lesions and the epidemiologic information relative to a recent excursion into the country, suggested the possibility of a pulmonary mycosis. Skin tests with histoplasmin[1] gave a 3-plus positive result in both cases, and with coccidioidin, positive for E. A. and negative for G. R. We inquired into the particulars of the excursion.

On February 6, 1954 a group consisting of 11 students, aged 14 to 18, and 2 instructors, aged 36 and 52, all residents of Barquisimeto, visited a cave situated approximately 50 kilometers to the south of the city, close to the town of Sarare in the Palavicino District of the State of Lara. The excursionists remained inside the cave for 2 to 3 hours and collected arachnids, pieces of stalactites and guano. Nine of them went down to the deepest part of the cave where there was a pool. In descending they were obliged to hold onto a rope, sliding over the rock. All these circumstances put them in close contact with the walls of the cave and the mud on the floor, while the intense exertion deepened and accelerated their respiration.

From 8 to 14 days later, five of them developed a moderate fever and general malaise. One presented a grippal condition with a fever lasting 5 days and a marked general weakness for several weeks. Two had fever for 15 days and more prolonged malaise. The remaining two were the students to whom we have already referred.

All the excursionists, with the exception of one who was not studied because he had changed his residence, responded with intense 3-plus reactions to histoplasmin and, with the exception of E. A., were negative to coccidioidin. Chest

[1] The coccidioidin and histoplasmin used in all the tests mentioned in this work were supplied by Drs. C. E. Smith, School of Public Health, University of California, Berkeley; and F. W. Cross, Division of Chronic Disease and Tuberculosis, Public Health Service, Washington D. C., respectively. The dilution used was always 1:100.

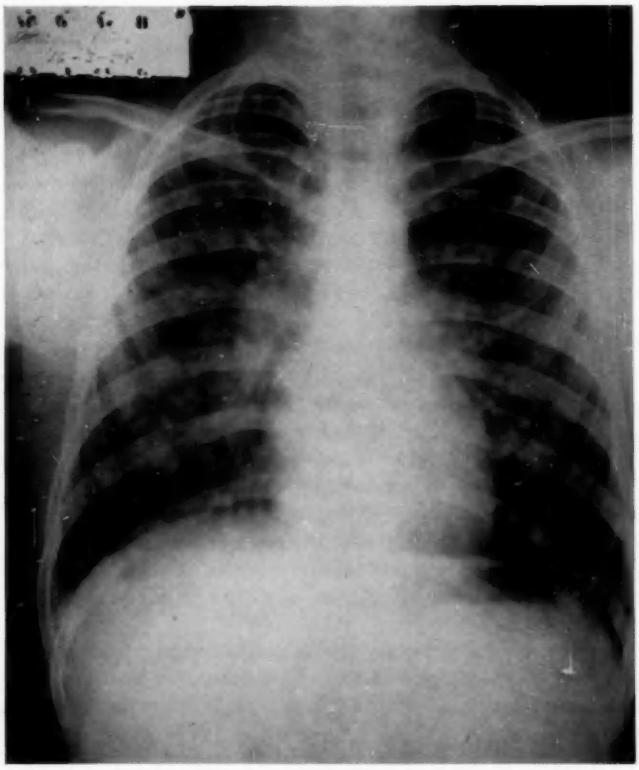

FIG. 1

radiographs of the three less intensely affected cases, made 6 weeks after the excursion, showed hilar thickening with nodular lesions more or less comparable to those described in G. R. and E. A. Of the seven remaining cases, five were normal and two presented the same appearance as the first three although with more discrete nodules. All but one gave histories of headache and asthenia for several days. The only one of the group whose health remained unchanged was one of the instructors, who had visited the same cave on previous occasions.

Complement fixation tests for histoplasmosis were made with the blood serum of eleven, through the cooperation of Dr. Libero Ajello and his collaborators in the Laboratory of Mycology, Communicable Disease Center, Chamblee, Georgia. Three were negative and were among the 5 persons whose x-rays were normal; one was anticomplementary; and seven were positive, with titers varying from 1:4 to 1:512. It is notable that the intensity of these reactions had no connection with the severity of the clinical picture nor with the extension of the x-ray shadows.

The epidemic form of the pulmonary infection of these persons; its spontaneous

regression in a relatively short time; the reactions, intense to histoplasmin and negative to coccidioidin; the results of the complement fixation tests; the absence of tubercle bacilli in the sputa and gastric contents, examined in the two cases most intensely affected—all of these conditions jointly sufficed to support a diagnosis of epidemic pulmonary histoplasmosis.

Now oriented, three of the authors, accompanied by the instructor who had been the guide for the student group, decided to visit the cave for the purpose of collecting material from which to try to cultivate the fungus. Prior to going, skin tests were made with the histoplasmin and coccidioidin. The histoplasmin gave 1 to 3 plus positive reactions in three of the members of the group, and one of them reacted positively to coccidioidin also. The fourth, M. J. D., did not react to either of the antigens. Radiograph films of the chest showed no active pulmonary lesions in any of the group. Upon leaving for the cave, on May 2, 1954, two more people joined the group. One had visited the cave several times before and the other was a resident of the region.

The point of entrance to the cave is situated at 300 meters above sea level, on the slope of a mountain covered for most part by tall growth. The mountain begins to rise at the banks of the Sarare River, in a valley of cultivated pasture-land, 270 meters above sea level. Rainfall in the area is copious during the rainy season and the average yearly temperature is 25°C., with extremes of 20°C. and 30°C.

Before entering the cave three members of the group, among them M. J. D., put on dust masks which the others refused. They passed into a large, completely dark cavern. Its walls were of rock, the ceilings and floors were very irregular because of the stalactite and stalagmite formations which capriciously divided the cavern into several sections or rooms in progressive descent. One of them opened into an almost vertical tunnel which led to the previously mentioned pool. The abundance of fish in the pool suggested a connection with the river.

The atmosphere was very humid and, in the pool zone, respiration was difficult. The temperature at 1:00 P.M. was 24.8°C. with a relative humidity of 100 per cent. The same determinations taken outside in the shade near the entrance to the cave were 25.4°C. and 92 per cent respectively. The cave harbors innumerable bats and some arachnids and its floor is covered with a thick layer of excrement dampened by filtrations of water. The group spent two hours exploring all of the passages, collecting samples from the floor of each. Only for a moment, while taking a snapshot, M. J. D. and another took off their masks, holding their breaths in the meantime.

Specimens were taken of powdered wood from the inside of the fallen trunk of a Mijaguo (*Anacardium rhinocarpus*), very close to the mouth of the cave, and 17 samples of soil were collected from the floors of the cavern's passages. A bat (*Vampirus noctiluca*) was also caught.

On May 18, 16 days after the visit to the cave, one of the authors, M. J. D., after suddenly developing a fever of 39°C., had headache, lumbar pain, pallor and general malaise. Several days later, he developed a dry intermittent cough, crepitations in the left lung base and slight splenomegaly, and chest x-rays taken on May 24 showed several hazy nodular infiltrations and moderate

bilateral thickening, findings which subsequently became intensified, but which diminished slowly until the majority of the lesions partially calcified in the course of the next two or three months.

Because of complete absence of expectoration, no sputum was obtained to be examined for the etiologic agent of the disease, but the diagnosis of histoplasmosis could be confirmed in this case by skin reactions to histoplasmin, since M. J. D. had been negative to this antigen on the eve of the excursion. A test with the same antigen, made two days after he became ill, produced a positive reaction, 10 mm. in diameter. Ten days later the result of a new test was 13 mm. in diameter and two weeks later a third provoked an intense reaction with erythema and infiltration measuring 30 mm. while the tests with coccidioidin remained negative.

The complement fixation test for histoplasmosis, performed in the same laboratory where those of the other excursionists had been made, gave a positive result at a titer of 1:128, with serum obtained 6 weeks after the onset of the disease.

STUDY OF MATERIAL COLLECTED IN THE CAVE

Histologic sections of the organs of the bat caught in the cave, studied by the pathologists at Hospital "Antonio Maria Pineda" of Barquisimeto, Venezuela, disclosed no pathologic alterations, and nothing was found in the wood dust. Microscopic examination of several suspensions of soil demonstrated some elements morphologically similar to clamydospores of *Histoplasma capsulatum*. In the laboratory of Mycology, Communicable Disease Center, Chamblee, Georgia, Dr. Libero Ajello and his co-workers obtained, by animal inoculation, four cultures of *H. capsulatum* from the five samples of soil that were sent them. This confirms the etiologic interpretation of the epidemic and demonstrates the abundance of the fungus in the cave.

SUMMARY

The serious illness, due to a pulmonary mycosis, of two members of a group which had been exploring a cave in the State of Lara, Venezuela, led to an examination of 10 other members of the group, all of whom gave positive reactions to histoplasmin, with confirmatory chest x-rays in 5 cases, and positive complement fixation tests in 7. Soil samples were taken from various parts of the cave, from 5 of which 4 cultures of *Histoplasma capsulatum* were obtained. In a second group of 5 visitors to the cave, one new case developed in the only member negative to histoplasmin prior to the visit, in spite of a respirator mask which he wore. The clinical and x-ray pictures, the appearance of complement-fixation antibodies, and the conversion of a negative to a positive histoplasmin reaction proved this to be a case of primary pulmonary histoplasmosis.

ACKNOWLEDGMENT

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REFERENCES

CAMPINS, H., AND SCHARYJ, M., 1950. Investigación de la sensibilidad cutaneo a la histoplasmina en Venezuela, *Arch. Venesol. Patol. Trop. y Parasit. Med.* **2:** 75–82.

CAMPINS, H., AND SCHARYZ, M., 1953. Comprobación de la histoplasmosis en Venezuela, *Gac. Med. de Caracas.* **60:** 67–75.

CHRISTIE, A., AND PETERSON, J. C., 1945. Pulmonary calcifications in negative reactors to tuberculin, *Am. J. Pub. Health.* **35:** 1131–1147.

FURCOLOW, M. L., AND GRAYSTON, J. T., 1953. Occurrence of histoplasmosis in epidemics. Etiologic studies, *Am. Rev. Tuberc.* **68:** 307–320.

FURCOLOW, M. L., AND LARSH, H. W., 1952. Direct isolation of *Histoplasma capsulatum* from soil, *Proc. Soc. Exptl. Biol. Med.* **80:** 246–248.

FURCOLOW, M. L., MANTZ, H. L., AND LEWIS, I., 1947. The roentgenographic appearance of persistent pulmonary infiltrates associated with sensitivity to histoplasmin, *Pub. Health Rep.* **62:** 1711–1718.

GORDON, M. A., AJELLO, L., GEORG, L. K., AND ZEIBERG, L. D., 1952. *Microsporum gypseum* and *Histoplasma capsulatum* spores in soil and water, *Science* **116:** 208.

GRAYSTON, J. T., AND FURCOLOW, M. L., 1953. The occurrence of histoplasmosis in epidemics. Epidemiological studies, *Am. J. Pub. Health* **43:** 665–676.

IBACH, M. J., AND LARSH, H. W., 1954. Direct isolation of *Histoplasma capsulatum* from soil, *Science* **119:** 71.

LOOSLI, C. G., PROCKNOW, J. J., TANZI, F., GRAYSTON, J. T., AND COMBS, L. W., 1954. Pulmonary histoplasmosis in a farm family. A three year follow up, *J. Lab. and Clin. Med.* **43:** 669–695.

PALMER, C. E., 1945. Nontuberculous pulmonary calcification and sensitivity to histoplasmin, *Pub. Health Rep.* **60:** 513–520.

POLLAK, L., 1953. Histoplasmosis en Venezuela. Ensayo epidemiologico, *Rev. Sanid. As. Soc. Venez.* **18:** 569–575.

POLLAK, L., RODRIGUEZ, C., AND ADRIANZA, M., 1953. Histoplasmosis pulmonar. Presentación de un caso, *Acta Medica Venezolana* **1:** 242–245.

PRIOR, J. A., AND COLE, C. R., 1951. Studies of the communicability of histoplasmosis, *Amer. Rev. Tuberc.* **63:** 538–546.

ZEIBERG, L. B., AJELLO, L., DILLON, A., AND RUNYON, L. C., 1952. Isolation of *Histoplasma capsulatum* from soil, *Am. J. Pub. Health* **42:** 930–935.

AN INTESTINAL PARASITE SURVEY IN THE HIGH CORDILLERAS OF PERU. I

E. H. PAYNE,[1] L. GONZALES-MUGABURU, AND E. M. SCHLEICHER

This is a report of a study done during early 1952 to determine the extent of intestinal parasitic infection in the mountain people of Peru. Throughout South America little is known regarding intestinal parasites among the inhabitants of the high Cordilleras. This program was sponsored by the Ministry of Health, Division of Communicable Diseases, of the Government of Peru, with financial support from the Department of Clinical Investigation, Parke, Davis and Company.

Callejon de Huylas was selected as the area in which to conduct the study. This narrow valley, drained by the Santa River, is 75 miles long. It begins at an elevation of 14,000 feet and ends at approximately 8,000 feet. From the western side of the valley rise the Black Cordilleras. On the east, covered with extensive glaciers and snow fields, are the White Cordilleras. This snow and ice furnishes an adequate supply of pure water to the valley, keeps the ground moist, and contributes, with the altitude, to keeping the climate at a temperate level. Historically the valley is the origin of one of the earliest cultures in South America which spread as far away as Easter Island.

In making the study of this area, nine groups, a total of 1305 subjects, were chosen from several different altitudes and from different sections of the population. Stool specimens were examined from a group of 58 people in Recuay, a village located at the highest altitude studied, approximately 11,000 feet above sea level. In Hauraz, at 10,000 feet elevation, we selected four groups. The first was a group of 233 unselected residents of the city; the second was a group of 169 mothers and children from the "Mother and Child" Welfare Center Clinic; 158 inmates of the Huaraz Jail, largely men from outside the valley, made up the third group; and 34 members of the Street Police, also from outside the valley, made up the fourth.

In order to study the indigenous population, 200 residents of a nearby plantation, Hacienda Vicos, were studied. These were all Quechua Indians living in primitive huts much as they had lived for centuries, and working at the plantation where the elevation was similar to that of the city of Huaraz.

At Carhuas, a town located at roughly 9,500 feet elevation, 79 residents were studied. At Yungay, several miles down the valley and at approximately 8,000 feet elevation, 195 were studied. A group of 179 were studied in Caraz the last town of any importance in the Callejon several miles beyond Yungay and practically the same elevation.

Thus, by working in one plantation and five villages at altitudes varying from 11,000 to 8,000 feet, we had a comparative study of subjects from different elevations as well as from both rural and urban areas. Methods of stool specimen

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examination were zinc sulfate concentration, fresh smear, and D'Antoni technique. The study of a greater number of specimens per individual would have been desirable, but study of two and in some cases three stool specimens for each individual were all we had time for.

Shortly after this study was started in January, 1952, it became obvious that the large number of parasites that were being encountered might be affecting the blood picture and/or liver function. Hematologic studies were done on the different groups and tests made to determine interference with liver function. To ascertain the latter Schleicher's (1951) method for detecting autohemag-

TABLE 1

Callejon de Huylas, Peru: intestinal parasite study

Percentages

Place	Recuay	Huaraz	CMI Huaraz	Carhuaz	Yungay	Caraz	*Huaraz* Jail	*Huaraz* Street Police	*Vicos* Quechua Indian
No. examined	58	233	169	79	195	179	158	34	200
Ascaris	13.7	12.4	12.0	41.5	25.9	19.0	25.9	2.9	82.0
Balantidium		2.8	0.7		1.0	1.1	5.2		4. 5
Blastocystis		8.0	2.3				2.5	26.4	
E. coli	65.5	66.0	74.0	73.0	78.9	76.0	86.4	65.4	88.0
Chilomastix	8.6	14.0	14.5	14.7	15.4	12.0	23.9	5.8	22.5
Endolimax	22.4	62.0	50.4	37.2	56.6	57.8	69.3	25.8	63.9
Fasciola		5.7	8.6		3.3	1.0	3.5	6.6	
Giardia	20.7	34.2	41.6	22.2	25.0	42.9	6.8	9.5	9.0
Hookworm							9.9	8.8	
Hymenolepis	12.0	17.1	7.3	17.2	15.8	13.4	7.3	2.9	5.0
E. histolytica	8.6	32.9	24.9	32.7	25.0	24.4	31.7	17.6	36. 6
Taenia		1.2				0.5	1.8		2.5
Iodamoeba	22.4	35.0	31.5	53.3	63.3	52.9	46.3	21.3	63.0
Heterodera		2.8	3.2	4.4	20.2	21.0	5.5		32.3
Trichocephalus	1.7	4.7	4.1	13.3	1.0	5.4	53.6	31.7	51.7
Trichostrongylus			1.2		1.0		0.6		0.5

TABLE 2

Eosinophile counts in 354 persons examined

Eosinophiles	Number	Eosinophiles	Number
%		%	
1	40	10	7
2	53	11	1
3	83	12	6
4	76	13	1
5	40	14	1
6	15	15	1
7	13	17	1
8	9	20	1
9	5	30	1

glutinins was used. This test was done on several hundred of the individuals studied, most of whom were selected for having heavy parasitic infection, especially a noteworthy number of *Fasciola hepatica*.

It was surprising to find that very few individuals showed any disturbance of liver function and those few cases that were found proved to have conditions other than the parasites which could explain this finding. Apparently the residents of this valley have lived so many years with their parasites that their physiology is adjusted to accommodate the infections.

Alterations in the blood picture did not prove to be significant. Eosinophilia was encountered in a number of the individuals examined, some showing as high as 30 per cent eosinophilia. No quantitative relation, however, could be established between parasitemia and the eosinophilia.

Hookworm infections were endemic in neighboring areas but the only hookworm infections found in Callejon de Huylas were found in those individuals who had entered the valley from those areas. While in the valley, an unsuccessful attempt was made to incubate the hookworm eggs gathered from these individuals. However, when these eggs were transported outside the valley to the coastal region, they were incubated without difficulty. At present there is no explanation offered for this observation.

Inhabitants of Callejon de Huylas enjoy a good diet. Beef and mutton are plentiful, and vegetables increase in amount as one descends the valley.

SUMMARY

Variation in altitude seemed to have little influence upon the percentages of parasites. The small number of persons studied in Recuay may account for the observed variations in this instance. Dwarf tapeworm was especially frequent and the first hundred persons examined all carried *Giardia lamblia*, which gave the investigators the impression (later proved false) of having found a population with 100 per cent incidence of this infection. *Heterodera* seemed to increase with lower altitudes, perhaps due to the increased consumption of vegetables of the collard family. A high incidence of *Ascaris* was noted among the Quechua Indians as well as of *Balantidium* which was also frequent in the jail inmates. Hookworm was present in the outlanders in the jail and in the police force. *Taenia* was not as common as expected and only one case of *Bodo* and one of *Enteromonas* were found, both among the Vicos people, and one case of *Haemonchus* at Carhuas. Four cases of *Isospora* and five of unidentified species of intestinal nematodes were found irregularly distributed throughout the valley. No correlation between the parasite population of an individual and his eosinophile count was discovered.

REFERENCE

SCHLEICHER, E. M., 1951. Erythrocyte aggregation factor in the plasma and serum of patients with acute lupus erythematosus, *Science* **113**: 558.

OBSERVATIONS ON *ASCARIS* DURING FLUOROSCOPY

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The presence of *Ascaris* infection is usually established by the demonstration of eggs in the stool, but since Fritz (1922) first described the procedure, such infections have frequently been recognized during fluoroscopic examination of the gastrointestinal tract. During the past 20 years the writer has performed over 12,600 gastrointestinal X-ray examinations, in about half of which evidence of *Ascaris* infection was recorded. The following observations upon the behavior of *Ascaris* in its normal habitat were made for the most part in 1952–53, when in the course of some 4,100 examinations, 1,937 infections were noted.

METHODS

Radiologic demonstration of *Ascaris* does not require methods basically different from those routinely used in fluoroscopic study of the gastrointestinal tract. In order to obtain the best visualization, however, the following technique is recommended:

a. The contrast medium, barium sulfate, is propelled from the stomach into the duodenum, and subsequently from the duodenum into the jejunum by digital pressure regulated through fluoroscopic observation.

b. Because of the movements of the worms, radiographic exposures must be short if well-defined pictures are to be obtained.

c. Success of the examination depends upon skillful digital manipulation which, to protect the operator, should be done quickly with the hands well shielded.

RESULTS

Distribution within the gut. Table 1 shows the distribution by site of 588 worms counted in 87 cases. In this series, 99 per cent of the worms were found in the jejunum and ileum, the proportion being about 8 to 1 in favor of the jejunum. Within the jejunum itself rather more worms were noted in the middle third than in either the anterior or posterior portions.

Table 2 shows the worm burden in the 87 cases reported above. In five persons, only a single worm was found. Three fourths of the group harbored from 3 to 10 worms and but a single patient carried more than 20. Worm burdens of the magnitude reported here seem to be characteristic of the urban population of Hiroshima.

Movement of Ascaris *within the gut.* Fluoroscopic examination furnished some interesting observations of the movement and behavior of *Ascaris* in its normal habitat. Almost all of the worms (92 per cent) were seen in the pose illustrated by Fig. 1. This position is neither affected by peristaltic movements nor is it altered by digital pressure through the abdominal wall. The worms resemble fish maintaining their position in a stream with the head pointing into the current.

TABLE 1

Distribution of 588 worms found in 87 cases of ascariasis

Site	No. of worms	%
Stomach	2	0.34
Duodenum	3	0.51
Jejunum (I)	173	29.4
Jejunum (II)	204	34.7
Jejunum (III)	138	23.5
Ileum	68	11.5
Total	588	

TABLE 2

Worm burden (same cases as Table 1)

No. of worms	No. of cases	%
Over 20	1	1.2
11–20	16	18.4
3–10	65	74.7
1	5	5.7
Total	87	

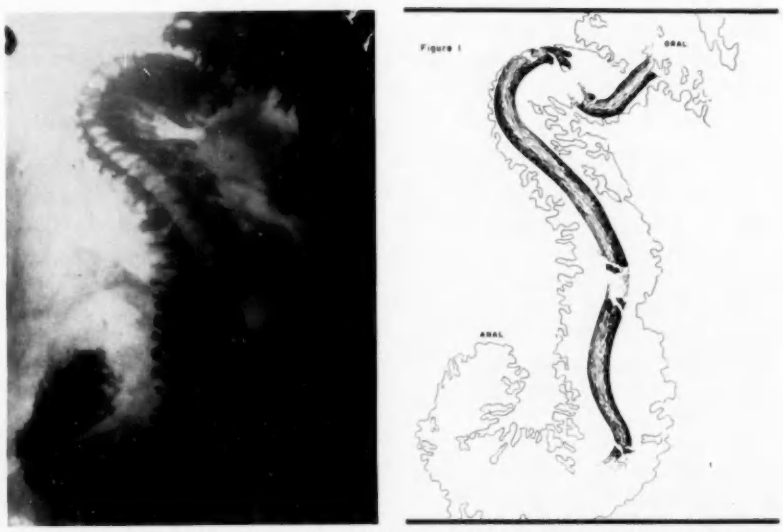

FIG. 1. Normal resting position. The slightly curved body of the *Ascaris* conforms to the contour of the intestine which harbors it.

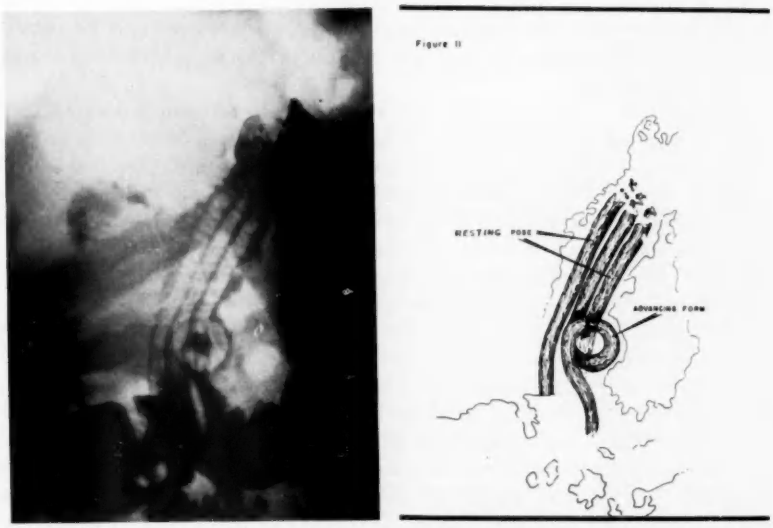

FIG. 2. Advancing pose. Three adult worms are in view. The one with a coiled body in the center is advancing; the two on either side are in the normal resting position.

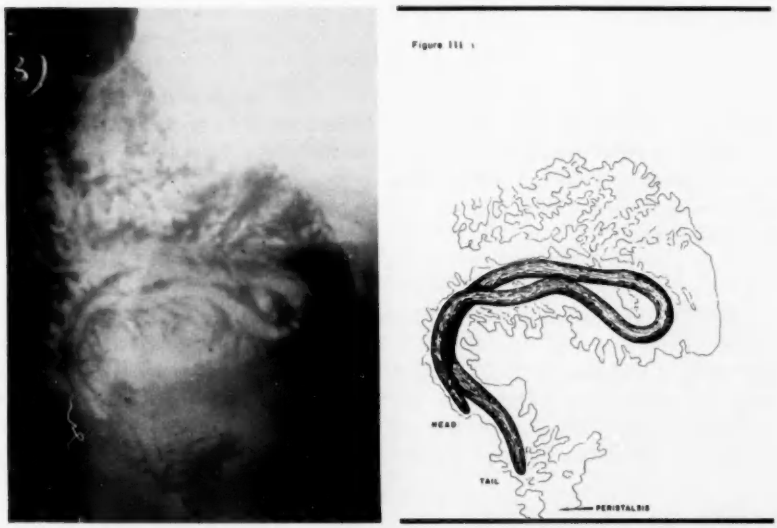

FIG. 3. Pose assumed after administration of anthelmintics. The worm is bent with the head and tail at approximately the same level, both pointing posteriorly.

In the presence of a large number of worms, X-ray pictures may be obtained without digital manipulation and the contour of the mass then may resemble a bundle of macaroni.

The ineffectual movements of *Ascaris* in a pan of water are in contrast to the positive progressive locomotion it exhibits in its normal habitat. Here it moves against peristalsis and movement of the bowel contents in a spiral fashion. The coiling of an advancing worm as it moves upward is shown in Fig. 2. Only about 5 per cent of the worms observed in this study were moving upward in the bowel.

When a worm moves downward in the gut it bends backward upon itself with the head moving downward after releasing its attachment to the intestinal mucosa. Less than 3 per cent of the worms observed were in this position.

Fig. 3 illustrates a position rarely seen in normal infections. Only two worms were noted in this position in 1,937 cases and were associated with gastric ulcer and gastric carcinoma respectively. However, the writer has frequently seen *Ascaris* assume this position following administration of an anthelmintic. When seen in this position with head and tail at about the same level pointing posteriorly, the worms are propelled passively through the intestinal lumen by peristalsis. Worms recovering from anthelmintics assume a normal posture and then move actively upward to those loops of bowel which constitute their preferred habitat.

Coupled forms have been seen only in the uppermost segments of the jejunum. Mature males and females of *Ascaris* can be easily identified during roentgenologic examination by the characteristic shape and curvature of the posterior end of the males.

SUMMARY

The distribution and behavior of *Ascaris* within the gastrointestinal tract has been observed by fluoroscopic methods. The jejunum is by far the favored habitat of *Ascaris*. A slight preference for the middle third over the anterior and posterior thirds is evidenced by the worms. Resting positions and locomotion within the bowel are described. A characteristic position is assumed following administration of anthelmintics.

ACKNOWLEDGMENT

The writer wishes to thank Dr. Emeric I. Dobos and Dr. R. H. Holmes of the Atomic Bomb Casualty Commission in Hiroshima and Dr. Frank H. Connell of the M. D. Anderson Hospital, University of Texas, for assistance in preparing these observations for publication. He is particularly grateful to Mr. Geoffrey Day also of the Atomic Bomb Casualty Commission for the illustrations he prepared from the writer's X-ray negatives.

REFERENCE

FRITZ, O., 1922. Askariden des Magendarmtraktes in Röntgenbild, *Fortschr. a. d. Geb. d. Röntgenstr.* **29:** 591–593.

FIBROSIS OF THE LIVER OF EGYPTIAN CHILDREN AND ADULTS[1]

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Fibrosis of the liver in both adults and infants has been reported from Egypt by Symmers (1904), Hutchison (1928), Hashem (1939), Dimmette (1954). These reports have usually focused attention on the role played by schistosomiasis in the production of fibrotic hepatic changes. Non-schistosomal forms were described by Hashem (1948) and have received very little further investigation or attention. Reports from India (Balasundaram, 1941; Veliaht and Strinivasachar, 1951; Mukerji, 1952); from Ceylon (Fernando *et al.*, 1948); from Southern Africa (Gillman and Gillman, 1945); from Iraq (Stacy, 1944); from West Africa (Roulet, 1946; Walters and Waterlow, 1954), and Eritrea (Ferro-Luzzi, 1949) indicate that infantile cirrhosis is a common pathologic state. This has often been labelled tropical cirrhosis and has also been reported from Jamaica (Royes, 1948), and South America (Ballon, 1948; Meneghello and Niemeyer, 1944). Review of the clinical records and pathologic reports indicates that the pathologic disease states are similar. The findings from surrounding as well as distant tropical and subtropical countries suggested the idea that non-schistosomal forms of fibrosis and associated changes in the livers should exist in many Egyptian children. This report is the result of a review of all of the clinical records and pathologic liver reports available to the Pathology Department of this Unit.

MATERIALS AND METHODS

One hundred and seventy-two cases were reviewed, and studied by wedge biopsy. Wedge biopsies were sufficient for cases proven to possess schistosomal infections by repeated urine and feces examinations. Twenty-two of the schistosomal infected cases were studied by multiple wedge biopsies of the liver in order to establish the pattern of fibrosis and the distribution of fibrous tissue in the various anatomic areas. The areas studied were as follows: Left lobe: anterior, posterior and middle parts. Right lobe: anterior, posterior, lateral and middle parts. Quadrate lobe: (only when resected in course of portovena caval anastomosis). All cases classified as non-schistosomal were studied at autopsy. Since the parasitic infection rate was high for both children and adults, complete autopsy tissues were utilized to establish the non-schistosomal cases.

The histologic studies were performed on formalin fixed tissues that had been routinely processed and stained with hematoxylin and eosin, Masson trichrome and Gridley's reticulum collagen methods.

[1] The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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RESULTS

The distribution of our cases is shown in Table 1. The ages varied from 6 months to 14 years for the children's group and from 14 to 64 years for the adult group. The average age for the children was 8.2 years. The average age for the adult group was 33.5. The male-female ratio for the children was 1.6:1 and 2.2:1 for the adults. Practically all of the cases came from rural and suburban districts of the delta in Egypt. Most of the patients over 6 years of age were farm workers. The general hygienic conditions were low and frequent diarrheal diseases and intercurrent infections were common in the history. Although the dietary histories were extremely difficult to evaluate, diets low in proteins and meats of all types were the rule. Since the group of cases were from rural areas, intermittent periods of basic protein and vitamin deficient intakes were probably frequent. These periods of deficient intake were interrupted by short periods of adequate intake of foods that were in season and could be obtained from local farms. In order to eliminate these dietary factors liver tissues were obtained over a period of two years, thus including two complete food cycles.

In Table 1, tabulations are shown for both children and adults. Further division has been made into schistosomal and non-schistosomal cases. In children the number of schistosomal cases with liver changes is too low to allow valid comparison with the non-schistosomal cases. Nevertheless, of the seven cases of schistosomiasis in children, four showed deposition of fibrous tissue in excess of normal. Two of these cases showed more than a minimal degree. In these cases inflammatory cell infiltration in the extralobular spaces was present, but only small amounts of fatty metamorphosis were seen in the parenchymal cells. The age of the parasitized patients varied from 6 to 13 years and sex distribution was 5 males and 2 females. Active untreated schistosomiasis was present in each patient with *S. mansoni* present in the bowel. *S. haematobium* was present in two of the male bladder walls.

TABLE 1

Distribution of cases used in wedge biopsies of the liver showing fibrosis, inflammatory cell infiltration, and fatty change

Age and type of case group	Total	Fibrosis					Inflammatory cell infiltration					Fatty change				
		0	+	++	+++	++++	0	+	++	+++	++++	0	+	++	+++	++++
Children, 0-13 yrs.																
Non-schistosomal	53	39	14	0	0	0	19	22	8	2	2	21	9	8	11	4
Schistosomal	7	2	2	1	2	0	0	4	1	2	0	4	2	1	0	0
Adults, 14 plus yrs.																
Non-schistosomal	25	15	5	3	1	1	6	9	6	2	2	23	2	0	0	0
Schistomal	87	10	31	30	14	2	6	35	31	13	2	73	9	5	0	0
Grand total	172	66	52	34	17	3	31	70	46	19	6	121	22	14	11	4
Subtotals																
Children	60	41	16	1	2	0	19	26	9	4	2	25	11	9	11	4
Adults	112	25	36	33	15	3	12	44	37	15	4	96	11	5	0	0

0 None; + Mild; ++ Moderate; +++ Marked; ++++ Severe.

TABLE 2

Analysis of non-schistosomal diseases in children with estimation of fibrosis, inflammatory cell infiltration and fatty change including cases of malnutrition

Primary disease present	Total	Degree of fibrosis					Inflammatory cells					Fatty change				
		0	+	++	+++	++++	0	+	++	+++	++++	0	+	++	+++	++++
Anemia	3	3						3				1		1	1	
Typhoid	15	13	2				4	6	3	2		7		3	4	1
Diphtheria	4	4					4					3	1			
TB meningitis	6	2	4				2	4				1	2	1	1	1
Pneumonia	10	5	6				4	5	2			1	5	1	3	1
Typhus	1	1						1				1				
Hepatitis	2	2							1		1	2				
TB (other than above)	4	2	2				1	1	2			2		1		1
Amebic dysentery	2	2					2					1		1		
"Ful" poisoning	2	2						2				1			1	
Pertussis	1	1					1								1	
Lymphoma	1	1									1	1				
Ricketts	1	1					1						1			
Total	53	39	14	0	0	0	19	22	8	2	2	21	9	8	11	4
Malnutrition also present	10	2	8	0	0	0	3	6	1	0	0	2	3	2	3	0

0 None; + Mild; ++ Moderate; +++ Marked; ++++ Severe.

TABLE 3

Analysis of non-schistosomal diseases in adults with estimation of fibrosis, inflammatory cell infiltration and fatty change including cases of malnutrition

Primary disease present	Total	Degree of fibrosis					Inflammatory cells					Fatty change				
		0	+	++	+++	++++	0	+	++	+++	++++	0	+	++	+++	++++
Anemia	1	1					1					1				
TB meningitis	2	2					2					2				
Pneumonia	1	1						1				1				
Hepatitis	4	1	1	1		1		1	1	2		4				
Amebiasis	5		4	1				2	2		1	5				
Lymphoma	3	2		1	1			1	1		1	2	1			
Laennec cirrhosis	1							1				1				
Vascular diseases	2	2					2					2				
Pulmonary abscess	1	1						1				1				
Acute sepsis	3	3						1	2			3				
Encephalitis	1	1						1					1			
Brain tumor	1	1					1					1				
Total	25	15	5	3	1	1	6	9	6	2	2	23	2	0	0	0
Malnutrition also present	9	3	4	2	0	0	2	4	2	0	1	8	1	0	0	0

0 None; + Mild; ++ Moderate; +++ Marked; ++++ Severe.

In the livers of children without evidence of schistosomal infection, fibrosis or collagen deposition was minimal in 14 of the 53 cases. No increase in fibrosis was seen in 39 or 73.5 per cent of the liver biopsies. The specific disease that caused or contributed to death has been listed in Table 2. From this table a representative spread of the diseases that we usually see in necropsies of children is present. Of all the diseases listed in Table 2, only typhoid, tuberculous, and pneumonic

cases showed any increase in fibrous tissue deposition in the liver. Of the fourteen cases with abnormal amounts of fibrosis, eight were accompanied by malnutrition that was evident at necropsy. In the typhoid cases, death was early in the course of the disease due to toxemia and malnutrition was not evidenced at autopsy.

Inflammatory cell infiltration in the liver showed a wide variation in the 53 cases of non-schistosomal diseases in children. The degree of inflammatory cell infiltration appeared to be related to disease entities and not directly to the fibrous tissue deposition as in the cases associated with schistosomal infections. Children with signs of malnutrition usually showed only a minimal degree of inflammatory reaction in the liver. The fatty changes in the parenchymal cells varied with disease intervals between onset and death. A correlation could not be established between fatty changes and fibrous tissue deposition. The cases with malnutrition showed marked fatty liver changes in 80 per cent.

The adult livers with schistosomiasis showed marked fibrous tissue deposition

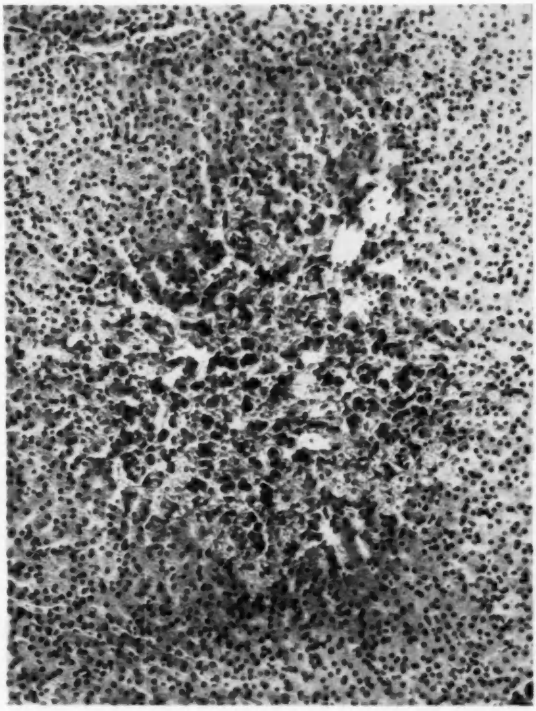

FIG. 1. Liver of child showing moderate degree of necrosis and mild inflammatory cell infiltration attributed to typhoid fever. × 187.

FIG. 2. Liver of an adult showing the absence of fat and moderate degree of fibrous tissue deposition. × 250.

and increased amounts of collagen. The correlation with inflammatory cell infiltration is shown in Table 1. Over 88 per cent of adult cases with schistosomiasis, both treated and untreated, showed abnormal amounts of fibrous tissue deposited in the liver. Only 16.1 per cent of the cases revealed fatty changes and these were usually minimal. The fibrous tissue was usually in the extralobular areas about the triads but lobular collagen and fibrous tissue were occasionally observed. Changes other than fibrosis, inflammatory cell infiltration and fatty metamorphosis in wedge biopsies of schistosomal livers have been described in detail in a previous report.

The non-schistosomal adult cases have been listed in Table 3 with the varying causes of death, which were similar to those seen in the children.

As for the distribution of fibrous tissue in the liver with schistosomiasis, fourteen cases with schistosomiasis ranging in age from 14 to 56 years were studied by multiple wedge biopsies of the liver. The sites used have been listed under materials and methods. The multiple sections failed to show any distinct con-

sistent degree of pre-election for any anatomic area. The anterior margins showed the greater number of parasitic forms and proved to be a better site for biopsy for etiological diagnosis. The fibrous acute angle along the leading edge of the anterior lobes was discounted for fibrous tissue evaluation. The distribution of fat as well as necrosis did not vary in the multiple sites biopsied.

The cases showed two types of patterns of fibrosis. In a minority of cases the fibrosis was restricted to the triads and about foci of egg forms. This resulted in a nodular type of pattern that has been described as classical for schistosomiasis. In the majority of cases the fibrous tissue deposition was in the perilobular areas surrounding each parenchymal cell group resulting in a smooth pattern. This type of fibrosis appeared related to the egg forms but extended into areas removed from the parasites to an equal degree. In these cases necrosis of parenchymal cells and extensive inflammatory cell infiltration were present. Collagen tissue and changes in the reticulum pattern were demonstrated. Encroachment of the fibrous tissue onto the parenchymal portion of

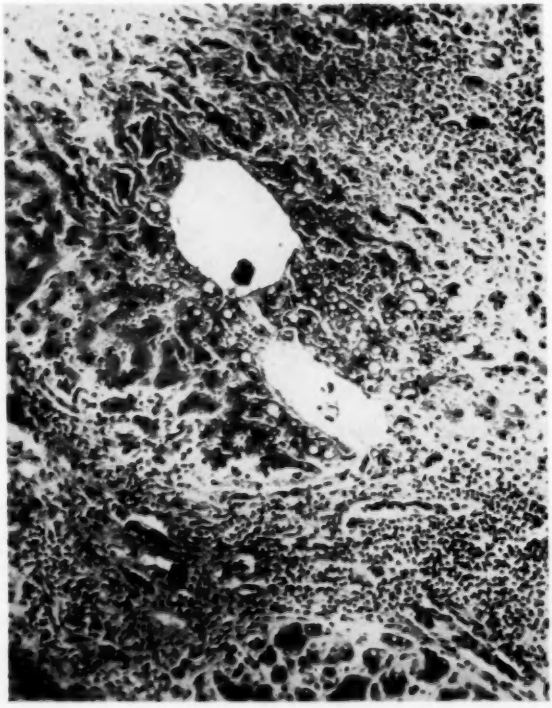

FIG. 3. Liver of an adult showing the marked degree of fibrosis associated with *S. mansoni* egg deposition. × 250.

FIG. 4. Liver of a young adult showing the nodular type of fibrosis frequently associated with schistosomiasis. Smooth types show little evidence of such a variated pattern. × 87.

the liver was obvious. These changes of both patterns have been described in a previous report from this Research Unit (Dimmette, 1954).

DISCUSSION

Many factors are believed to be at play in the production of fibrosis in the livers of Egyptians. The degree of parasitism by schistosomiasis alone is striking. This has been shown to produce fibrotic changes in the livers. Karsner (1943) refers to this type of fibrosis as not a true cirrhosis. Nevertheless, it fulfills the definition as established by Mallory (1911) and reiterated by Davidson (1954), yet regeneration changes have not been observed. At this Research Unit we have been impressed by the absence of regenerative changes in the liver in cases of fibrosis associated with schistosomal infections. Mixed cases of viral hepatitis are occasionally seen associated with schistosomiasis with marked fibrosis of the liver. The changes attributed to each of the diseases can usually

be separated even if at times with difficulty. These cases have not been included in this study because of their complex nature.

Other forms of parasitism are commonly present in Egyptian autopsy material. Frequent infections by *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Trichuris trichiuria*, and *E. histolytica* are observed. Occasional cases of filariasis and malaria have been seen, but are rare in the regions of Egypt from which this material was obtained. Minor degrees of hepatic fibrosis could possibly be attributed or associated with these parasitic infections but apparently do not play an important role as pointed out by Stacy in Iraq (1944).

On the other hand, malnutrition is the rule in most of the cases subjected to study and it is apparent that this factor is of importance. From observations of liver sections, the cases in Egypt are similar to the cases of nutritional diseases in other regions of East and North Africa. The nutritional factors are believed to be of importance in both the parasitized cases as well as those free of schistosomal infections. The role played in the schistosomal cases is believed to be greater than previously assessed. There is little doubt that the combination of schistosomal infections of the liver and malnutritional states contribute to the massive degrees of fibrosis of the liver observed in Egyptians. This combination deserves further investigation and study. Additional studies (histologic and biochemical) are in progress at this Unit to evaluate further the changes in the liver of Egyptian children both parasitized and free of schistosomal infections. Corrective diets are being used and serial needle biopsies of the livers are under study.

The abrupt changes in fat deposition in the liver at about age fourteen years require further explanation. The adult livers contain very little fat. Of 112 adult livers examined only five cases showed fat in excess of normal; whereas 35 of the 60 children's livers contained excess fat. Twenty-four of these cases had more than a minimal degree. From the dietary histories, it is apparent that changes in food consumption, both quantity and quality, occur about puberty. Children at puberty, no longer confined to the house and courtyards in the villages, are placed in the agricultural fields as workers. If they are not employed in farm jobs, the occupational trades bring monetary returns and they emerge as providers. When a provider status has been acquired, restrictions on diet that may have been imposed on them as children are removed. Proteins including meats and milk products are ingested in quantities believed to be sufficient to remove fat from the liver. The incidence of active schistosomal infections does not decrease at this age as shown by previous studies (Dimmette *et al.*, 1955), and exposure to schistosomal cercaria may be increased. Nevertheless, the adult frequents the treatment centers more often and receives specific therapy for schistosomal infections as well as other forms of parasitism.

The close relationship of inflammatory infiltration in the extralobular areas and fibrosis has been stressed in a previous report (Dimmette, 1954). This observation was made on adult livers and patients harboring visceral forms of schistosomiasis. The current study indicates that a like correlation can be ap-

plied to children. Nevertheless, the inflammatory cell infiltration and fatty change are not closely correlated in the non-schistosomal cases. If hepatitis and lymphomatous cases are not considered in Tables 2 and 3, the relationship is further separated. The tabulations also indicate that a higher percentage of children's livers show an excess number of inflammatory cells than fat changes. In the adult this percentage difference is marked. In several of the non-schistosomal livers that possessed excess collagen and fibrous tissues, mononuclear inflammatory cells were common, yet their exact significance could not be determined. Moschowitz (1948) concluded that the beginning of cirrhosis in the fatty liver is represented by round cell infiltrations of the periportal spaces. Though common in children as emphasized by Gillman and Gillman (1945) studying infantile pellagra, the significance of such infiltrations has been minimized or overshadowed by the great interest in fibrous tissue deposition in the same livers.

From the available case histories on living patients that were included in this study, the frequency of treatments for hookworm (*Ancylostoma duodenale*) was estimated at every two to three years on numerous children and adults. Although other drugs are used for this parasitic infection, carbon tetrachloride remains in popular use at treatment centers. This drug is well known for its toxic, necrotizing, fat producing and fibrosing changes in the liver. Although the dosages are kept within the accepted therapeutic ranges as based on weight as well as age, the frequent treatments in the poorly nourished or borderline case of malnutrition could be considered as a fibrosing hepatic agent. This phase, as well as therapeutic industrial agents toxic to the liver, has not been explored to any great extent in Egypt. Industrial chemicals, agricultural agents, or others of the known liver toxins probably do not play an important role in Egypt. Tetrachlorethane is used in small amounts in Egypt as a therapeutic agent and is considered to be a liver toxin followed by fibrosis. Hashem (1939) listed this drug as a possible etiological agent in children but concluded that the cirrhosis of children was due to "metabolic toxins of gastro-intestinal origin.'

SUMMARY

One hundred and seventy-two livers from Egyptian children and adults were studied by biopsy for fatty change, inflammatory cell infiltration and fibrosis. Sixty children, seven of whom had visceral forms of schistomiasis were included. Besides fatty changes and inflammatory cells in excess of normal, 32 per cent of the children showed varying degrees of fibrosis, but over 76 per cent of the children with schistosomiasis or signs of malnutrition showed excess fibrous tissue deposition. Abrupt changes in fatty metamorphosis observed about the age of puberty are attributed to marked dietary changes instituted at this age.

Of the adults, 78 per cent had schistosomiasis, and this had a profound effect upon fibrosis of the liver, producing both nodular and smooth types. But again, as in the children, malnutrition, whether sustained or interrupted, was probably

the most important etiological agent responsible for fibrosis in the absence of schistosomiasis. Factors other than parasitism and malnutrition are believed to be unimportant.

REFERENCES

BALASUNDARAM, S., 1941. Infantile biliary cirrhosis; symptoms, pathology, diagnosis and treatment, *Indian M. J.* **35:** 292–296.

BALLON, O. U., 1948. Contribution a la histo-patologia de las cirrosis y seudo-cirrosis hepaticas, *Arch. Peruanos Path. Y Clin.* **2:** 3–71.

DAVIDSON, C. S., 1954. Cirrhosis of the liver, *Amer. J. Med.* **16:** 863–873.

DIMMETTE, R. M., 1954. The comparison of wedge and needle biopsy of the liver in schistosomiasis in Egyptians, *Gastroenterology*, **29:** 219–234.

DIMMETTE, R. M., SPROAT, H. F., AND KLIMT, C. V., 1955. Examination of urinary sediment for the diagnosis of neoplasms of the bladder. A survey of an Egyptian village infested with *S. haematobium*, *Amer. J. Clin. Path.* **25:** 1016–1042.

FERNANDO, P. B., MEDONZA, O. R., AND RAJASURIYA, P. K., 1948. Cirrhosis of the liver in Ceylon and its relation to diet, *Lancet*, **255:** 205–211.

FERRO-LUZZI, G., 1949. La cirrosi atrofica del fegato in Eritrea, *Boll. Soc. Ital. Med. e Ig. Trop.* **9:** 223–236.

GILLMAN, T., AND GILLMAN, J., 1945. Hepatic damage in infantile pellagra, *J.A.M.A.* **129:** 12–19.

HASHEM, M., 1939. Aetiology and pathology of types of liver cirrhosis in Egyptian children, *J. Roy. Egypt. Med. Assoc.* **22:** 319–354.

HASHEM, M., 1948. The incidence of non-schistosomal forms of hepatic cirrhosis in Egypt their morphology and pathogenesis, *J. Roy. Egypt. Med. Assoc.* **31:** 222–229, 541–555, 635–650.

HUTCHISON, H. S., 1928. The pathology of bilharziasis, *Am. J. Path.* **4:** 1–16.

KARSNER, H. T., 1943. Morphology and pathogenesis of hepatic cirrhosis, *Amer. J. Clin. Path.* **13:** 569–606.

MALLORY, F. B., 1911. Cirrhosis of the liver, five different types of lesions from which it may arise, *Bull. Johns Hopkins Hosp.* **12:** 69–75.

MENEGHELLO, J., AND NIEMEYER, H., 1944. Cirrosis hepatica en il niño, *Rev. Chilena de Pediat.* **15:** 126–136.

MOSCHOWITZ R., 1948. Laennec cirrhosis, *Arch. Path.* **45:** 187–215.

MUKERJI, S. K., 1952. Infantile cirrhosis of liver, *J. Indian M. Asso.* **22:** 6–12.

ROULET, VON F., 1946. Über die Zeberzirrhose der schwarzen Bevolkerung Afrikas, *Schweiz. Ztschr. f. Path. u. Bakt.* **9:** 666–669.

ROYES, K., 1948. Infantile hepatic cirrhosis in Jamaica, *Caribbean M. J.* **10:** 16–48.

STACY, R. S., 1944. Portal cirrhosis in Iraq, *Tr. Roy. Soc. Trop. Med. & Hyg.* **37:** 387–398.

SYMMERS, W. ST. C., 1904. Note on the new form of liver cirrhosis due to the presence of ova of bilharzia haematobia, *J. Path. & Bact.* **9:** 237–239.

VELIAHT, G. D., AND STRINIVASACHAR, S., 1951. Cirrhosis of the liver in Madras Province, South India, *J. Indian M. A.* **20:** 271–274.

WALTERS, J. H., AND WATERLOW, J. C., 1954. *Fibrosis of the liver in West African children*, Med. Res. Council Spec. Rep. Ser. No. 285. H. M. Stationery Office, London, pp. 1–71.

STUDIES ON SCHISTOSOMIASIS

XII. Some Ointments Protecting Mice Against the Cercariae of *Schistosoma Mansoni*[1]

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The importance of the control of schistosomiasis is self-evident. Most control measures are not practical for use by the military. At present two possibilities exist: (1) the use of protective clothing; and (2) a protective ointment to apply to the exposed skin. The progress made in these two fields has been summarized to 1953 and will not be repeated here, although the reports of Ferguson *et al.* (1946), Wright *et al.* (1948), and Kemp and Hunter (1955) should be mentioned. Recently Moon and Hunter (1954, 1955) reported on the failure of copper oleate to protect white mice against the penetration of cercariae of *Schistosoma mansoni* although this ointment was effective against *S. japonicum*. Radke, Hunter *et al.* (1953, 1954) tested four others; none was completely satisfactory. Subsequently in 1953 support was given to this program by the Armed Forces Epidemiological Board. During 1953-54, 134 compounds were screened by means of "preliminary screening tests" (Kemp, Hunter *et al.*, 1954; Kemp and Hunter, 1955). Many of these were highly promising. Bauman *et al.* (1954, 1955) reported on the effectiveness of a repellent cream (Abbott E-4856) in protecting mice against *S. mansoni* cercariae. In 1954–55 the work on protective ointments was continued, resulting in the findings reported herewith.

MATERIALS AND METHODS

Preparation of ointments. The preparation of potential protective ointments was carried out according to the previously described method (Kemp, Hunter *et al.*, 1954, (1956); Kemp and Hunter, 1955) except that wool fat (anhydrous-*adeps lanae* U.S.P.) was substituted for petrolatum as an ointment base. This change was made because petrolatum was unsatisfactory.

The snail colony and infected stock. The maintenance of the infected snail colony, the technique of obtaining cercariae from these infected snails, and the method of infecting the mice have been described previously (Kemp and Hunter, 1955). The perfusion technique for laboratory animals utilized the further simplification of Pan and Hunter's (1951) modification of the original prefusion method described by Yolles, Moore *et al.* (1947).

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FIG. 1. Plastic infection boards with mice in position over the infection wells

In vivo experiments. Preliminary screening tests. This series of tests initiated the in vivo experiments. The purpose was to screen out the ointments showing cercaricidal properties as applied to mice under laboratory conditions. Mice were shaved with clippers, taped down on infection boards (Fig. 1) with the "ointment-protected" surface of the mouse in contact with the surface film of water in the well. One hundred pooled cercariae were added and the protected and control mice were exposed for 30 minutes each.

The original plan was to screen each ointment by testing two series of about 15 mice each so that each "protected" group would total at least 25 mice. Since time was not available to screen so many ointments by this technique it became necessary to use only 15 mice per test. During the final phase of the program it sometimes became necessary to submit the mice directly to the more critical "four hour immersion test" without the benefit of the preliminary screening tests (Kemp and Hunter, 1955).

"Four-hour immersion test" with Schistosoma mansoni. A battery of four five-gallon aquaria was used in this experiment. A continuous flow of water at a level of approximately 1 to 1½ inches was maintained by means of brass inlet and outlet tubes (Fig. 2). The outlet tubes were connected to a Richards' Suction Pump, thus facilitating the maintenance of a constant water level. The "protected" and control mice were placed in separate aquaria where they were bathed constantly in water varying between 38° and 40°C. during the four hour test period. The mice were then dried under warm air fans, and each was exposed to 100 cercariae of *S. mansoni* for 30 mniutes. They were then air dried and held for six weeks before autopysing to determine the number of adult schistosomes present per mouse. All of these procedures have been previously described (Kemp and Hunter, 1955).

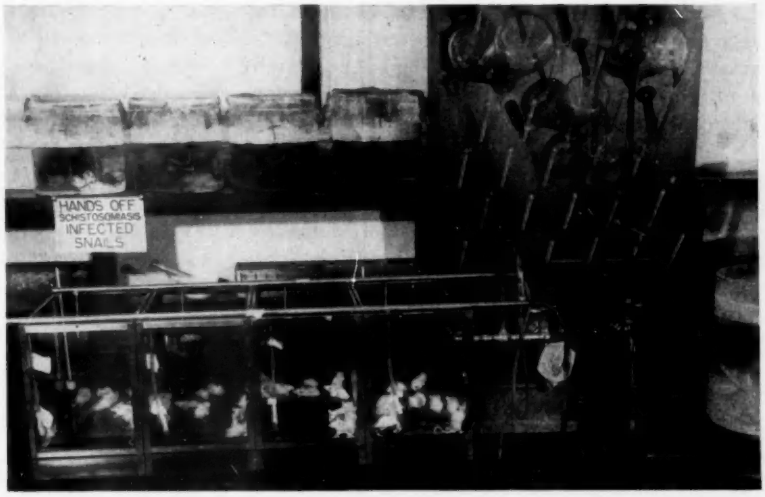

FIG. 2. Battery of five gallon aquaria used in the four hour immersion tests

"Four-hour immersion test" with S. japonicum. These experiments were carried out exactly as for *S. mansoni*, except that only 25 to 50 pooled cercariae of *S. japonicum* were available per mouse.

In vitro experiments. The best ointments were subjected, along with their constituent parts, to in vitro tests. These tests consisted of "contact" and "diffusion" tests. The "contact" test consisted of (1) "immediate contact tests" to determine whether or not ointments were immediately cercaricidal, and (2) "delayed contact tests" to ascertain cercaricidal property of ointments after contact with water for 24 hours.

In the *immediate contact test*, two embryological watch glasses were smeared with a film of the ointment under test. These preparations were allowed to air dry for 30 minutes to insure the evaporation of possible volatile agents. Five ml. of aerated chlorine-free water was added to the ointment-coated watch glasses and to each of three control watch glasses. Twenty cercariae of *S. mansoni* were added to each of these dishes and observed under a dissecting microscope to determine the number of viable cercariae at the end of 15, 30, 45, 60, and 120 minutes.

In *"delayed contact tests"* the same procedure was followed, except that the compounds in question were allowed to remain in contact with aerated water for 24 hours before the introduction of cercariae. During one-half of the contact period, the water in each test dish was changed at two-hour intervals in order to remove any of the active ingredients that might diffuse into the water. At the end of 24 hours a final change of aerated, chlorine-free water was made and 20 cercariae were introduced to each of the watch glasses, which were observed at the end of 15, 30, 45, 60, and 120 minutes unless all cercariae were killed sooner.

Diffusion tests. These were instituted to find out whether the active principle of the ointments used would diffuse into water in sufficient concentration to kill the cercariae of *S. mansoni* at the end of four and 72 hours. In the four-hour diffusion tests the embryological watch glasses were smeared with a thin film of the compound being tested. Five ml. of aerated, chlorine-free water were added to each of the test dishes and allowed to stand for four hours with two minutes of agitation per hour. At the end of four hours the fluid from each of the test containers was filtered into a clean watch glass and five ml. of aerated, chlorine-free water was added. Twenty cercariae of *S. mansoni* were introduced to each of the test and control watch glasses and observed for viable and dead cercariae as described for the delayed contact tests (*vide supra*). Except for the increased time in exposure of the ointments to water, the 72-hour diffusion tests were carried out in the same manner as the four-hour tests.

In expressing the results of the in vitro tests, the form of a fraction was used (Table 4). For instance, the figure 20/15 indicates that 20 cercariae died within 15 minutes after being introduced to a container coated either with a thin film of test ointment or a water filtrate from a test ointment.

Materials that were selected as potential ointment bases were also subjected to in vitro tests in order to determine whether or not they were toxic per se to cercariae of *S. mansoni*.

Toxicity tests. Ointments providing a relative per cent protection of 99–100 were selected for semi-qualitative toxicity tests. These tests were carried out primarily to determine the effect of repeated topical applications (i.e., 15) of ointments on the survival of white mice during a three week period. Twenty test mice and 30 control mice were used in each semi-qualitative toxicity test. Ointments were applied to the shaved belly surfaces of the test mice in as nearly as possible the same amounts as used in the in vivo tests. During this three week period, daily records were kept on mortality and general health of the mice.

RESULTS

Preliminary screening tests had been conducted on 134 ointments to determine which warranted further testing as potential protctive agents against the penetration of cercariae of *S. mansoni* (Kemp *et al.*, 1954; Kemp and Hunter, 1955). Of these, 85 preparations gave complete protection and so were selected for further testing by the four hour immersion test. In addition, another 42 ointments were tested without the use of the preliminary screening tests. Thus, a total of 127 compounds were subjected to the rigorous four hour immersion test utilizing white mice as the test organism.

Four-hour immersion tests on S. mansoni. Eighteen ointments afforded 99 to 100 "Per Cent Relative Protection" (PRP) after four hours immersion in water (Table 1). Fifteen other ointments provided 90 to 98.9 PRP (Table 2), while the remaining 94 provided less than 90 PRP (Table 3). The average number of worms recovered per control mouse was 37.8 with a range of 8.8 to 56.7 (Tables 1, 2, 3).

Four-hour immersion test on S. japonicum. Three tests were carried out with

TABLE 1

Ointments affording 99–100 per cent relative protection for 30 minutes against 100 cercariae of Schistosoma mansoni *following the four-hour immersion test*

Ointment and per cent active ingredient*	Ratio of mice "protected" (at autopsy)†	Average number of worms per mouse (at autopsy)		Per cent relative protection‡
		"Protected" mice	Control mice	
1. Actamer (10); Silicote	49/49/55	0.0	44.6	100
2. Actamer (5); Hexachlorophene (3); benzyl benzoate (20); wool fat	40/40/50	0.0	37.6	100
3. Benzyl benzoate (20); Covicone with repellents	44/44/55	0.0	37.0	100
4. Hexachlorophene (10); wool fat§	6/6/10	0.0	56.7	100
5. Hexachlorophene (5); wool fat	30/30/34	0.0	41.1	100
6. Hexachlorophene (5); benzyl benzoate (20); copper stearate and copper oleate in ether	41/41/52	0.0	49.4	100
7. Hexachlorophene (5); copper stearate (40); copper oleate in ether	42/42/52	0.0	40.0	100
8. Hexachlorophene (3); Covicone with repellents	40/40/50	0.0	25.1	100
9. Hexachlorophene (10); olive oil (10); petrolatum	16/16/17	0.0	23.4	100
10. Hexachlorophene (3); Pro-derna	40/40/52	0.0	40	100
11. Actamer (5); olive oil (5); wool fat	56/57/67	0.014	36	99.9
12. Actamer (5); Pro-derna	45/46/54	0.02	42.6	99.9
13. Actamer (5); n-butyl acetanilide (5); dimethyl phthalate (5); Indalone (5); wool fat	38/39/53	0.05	50.8	99.9
14. Benzyl benzoate (20); dibutyl phthalate (10); wool fat	49/52/55	0.06	49.4	99.8
15. Captan (5); wool fat	49/52/59	0.07	42.7	99.8
16. Benzyl benzoate (20)§; Hexachlorophene (10); wool fat	7/8/10	0.12	33	99.6
17. Hexachlorophene (1); wool fat	53/57/63	0.28	45.2	99.3
18. Benzyl benzoate (10); Indalone (5); dibutyl phthalate (5); dimethyl phthalate (5); wool fat	21/23/54	0.26	35.8	99.2

* The percentage of the component is the number in the parentheses—(10); commercially prepared ointments are indicated by "(−)."

† This is expressed as 10/12/14; i.e., 10 of 12 mice surviving to autopsy were negative; the last figure(14) shows the total number protected mice infected.

‡ Relative per cent protection indicates the *degree* of infection in the "protected" mice. It is calculated as follows: where the average number of worms recovered from control mouse is x, the average number of worms from "protected" mouse is y, and the relative per cent protection is z; then $z = (x - y/x)$. Thus, $x = 45$, $y = 35$, $z = (45 - 35/45) = 22.2\%$ relative protection.

§ Preparation highly toxic at this concentration.

TABLE 2

Ointments providing 90–99 per cent relative protection for 30 minutes against 100 cercariae of Schistosoma mansoni *following the four-hour immersion test*

Ointment and per cent active ingredient	Ratio of mice "protected" (at autopsy)	Average number of worms per mouse (at autopsy)		Per cent relative protection
		"Protected" mice	Control mice	
1. Actamer (5); Ply-3	34/43/55	0.6	28.6	98.9
2. Actamer (5); copper oleate in ether	6/10/10	0.7	53.0	98.7
3. Spergon (5); Pro-derna	8/9/10	0.74	39.1	98.1
4. Benzyl benzoate (10); Silicote	6/10/10	0.9	44.0	97.9
5. Actamer (5); West #311	7/8/10	0.25	43.0	97.2
6. Copper-8-quinolinolate (5); olive oil (5); petrolatum	6/9/10	0.55	27.8	97.0
7. Spergon (5); wool fat	38/50/54	1.3	37.1	96.4
8. Actamer (5); olive oil (5); petrolatum	17/20/20	0.25	31.5	96.0
9. Covicone with repellents (−); 1 application	12/26/31	1.6	28.6	94.7
10. Actamer (5); dibutyl phthalate (5); benzyl benzoate (20); copper oleate in ether	25/48/55	2.2	38.4	94.2
11. Benzyl benzoate (20); Prantal	15/16/20	2.2	36.1	93.9
12. Benzyl benzoate (10); wool fat	2/6/10	1.5	38.4	93.4
13. Benzyl benzoate (26.5); Aqueous	37/49/57	2.3	27.3	93.4
14. Benzyl benzoate (10); Tween 60 (1); Aqueous	5/8/10	2.87	35.5	91.6
15. Cunilate #6249 (−)	8/14/20	3.14	48.8	91.1

See footnotes, Table 1.

S. japonicum, but the results were equivocal because of the small number of cercariae of *S. japonicum* available for each test.

In vitro tests. In vitro tests were carried out on all of the best ointments and their constituent ingredients. Most of the ointments listed in Table 1 were so tested. With the exception of Silicote and Pro-derna, which killed all cercariae within 15 minutes in immediate contact tests, ointment vehicles and levigating agents had no effect on cercariae. Copper stearate, dibutyl phthalate, dimethyl phthalate, Indalone and n-butyl acetanilide were likewise ineffective as cercaricidal agents. On the other hand, such agents as benzyl benzoate and the combination of chemicals used in the 18 successful ointments revealed a high degree of activity against the cercariae of *S. mansoni* (Table 4).

Toxicity tests. The survival of test mice ranged from 10 to 100 per cent when subjected to 15 topical applications of the test ointment in comparison with the controls which fall between 90 and 100 per cent. In some cases the abdominal skin on the test mice revealed macroscopic changes ranging from a slight hardening of skin to a very rough, scabrous surface, occasionally even with open lesions. One batch of test mice treated with Actamer, Hexachlorophene, and benzyl benzoate in wool fat, had cervical swellings. Some of these became pustular and burst, exuding pus and blood. Whether or not the latter condition was due to

TABLE 3

Ointments providing less than 90 per cent relative protection for 30 minutes against 100 cercariae of Schistosoma mansoni *following the four-hour immersion test*

Ointment and per cent active ingredient	Ratio of mice "protected" (at autopsy)	Average number of worms per mouse (at autopsy)		Per cent relative protection
		"Protected" mice	Control mice	
1. Aero lubricant (−)	0/28/30	37.2	37.7	0.0
2. Amertan (−)	0/8/9	99.2	29.4	0.0
3. Aramite (5); olive oil (5); petrolatum	0/8/10	17.4	28.5	38.9
4. Arquad C (5); petrolatum	0/8/10	63.2	39.5	0.0
5. Arquad 2C (5); petrolatum	0/8/10	47.5	39.5	0.0
6. Arquad 2C (5); wool fat	0/7/9	19.8	37.6	47.3
7. Arquad 18 (5); petrolatum	0/6/9	55.5	39.5	0.0
8. Arquad 18 (5); wool fat	0/9/10	53.7	37.6	0.0
9. Arquad S (5); petrolatum	0/5/10	43.2	41.7	0.0
10. Arquad T (10); petrolatum	1/9/10	21.0	41.7	49.6
11. Arquad T (10); wool fat	0/8/10	37.3	18.8	0.0
12. B.A.L. (−)	4/13/20	10.7	13.0	17.0
13. B.A.L. (50); Covicone with repellents	0/10/10	41.0	35.3	0.0
14. Benzyl benzoate (1); Tween 60 (1); Aqueous	6/10/10	8.5	35.5	76.9
15. Benzyl benzoate (5); Tween 60 (1); Aqueous	2/10/10	4.5	35.5	87.1
16. Benzyl benzoate (10); Tween 80 (1); petrolatum	1/8/10	25.8	48.1	46.3
17. Benzyl benzoate (20); Hexachlorophene (10); Tween 60 (1); in H_2O*	—	—	—	—
18. Benzyl benzoate (20); copper oleate (40); in ether	11/35/40	16.2	39.0	58.5
19. Benzyl benzoate (10); Indalone (5); dibutyl phthalate (5); dimethyl phthalate (5); Tween 60 (5); Aqueous	0/7/10	34.2	38.0	10.0
20. Benzyl benzoate (20); Kerodex 71	2/10/10	20.4	35.3	42.2
21. Benzyl benzoate (21.5); Santocel (10); olive oil	2/18/20	11.8	45.4	74.0
22. Benzyl benzoate (10); Santocel (2); in wool fat	4/9/11	4.6	38.4	88.0
23. Breck pH 7 (−)	1/16 22	35.1	42.3	17.0
24. Captan (5); olive oil (5); in petrolatum	0/6/10	18.5	28.5	35.0
25. Captan (5); in Prantal	0/8/10	25.8	43.0	39.8
26. *C. foetidissima* extract (10); in wool fat	0/8/10	27.7	42.4	34.6
27. Cello-Seal (−)	0/21/25	39.6	40.1	1.22
28. Copper oleate (90); and Indalone	0/17/20	18.1	43.9	58.7
29. Copper oleate (50); copper stearate (50)	0/17/20	7.9	29.6	73.3
30. Copper NN[1] dibutyl ethylene diamine sodium complex (5); olive oil (5); in petrolatum	0/7/10	41.2	27.8	0.0

See footnotes, Table 1.

* All test mice dead within 24 hours.

† 1 application per day for 10 days.

‡ 10 applications every one-half hour for 10 applications.

TABLE 3—*Continued*

Ointment and per cent active ingredient	Ratio of mice "protected" (at autopsy)	Average number of worms per mouse (at autopsy)		Per cent relative protection
		"Protected" mice	Control mice	
31. Covicone with repellents†	0/21/30	32.8	60.0	45.4
32. Covicone with repellents‡	0/28/29	18.3	48.4	61.9
30. Covicone M-20-20	0/42/50	24.4	44.7	45.5
34. CP 6419 (5); olive oil (5); in petrolatum	2/15/20	7.15	17.0	59.6
35. CP 7150 (5); olive oil (5); in petrolatum	2/17/18	7.4	20.4	63.0
36. CP 7151 (5); olive oil (5); in petrolatum	0/18/20	37.7	37.6	0.0
37. Cunilate lotion emollient (10); wool fat	26/45/57	11.1	45.0	75.3
38. Cuniphen #6200 ointment (−)	0/18/18	20.8	42.4	50.9
39. Desenex (−)	0/6/9	55.6	33.1	0.0
40. Dial Soap (−)	0/10/10	37.0	45.2	18.1
41. Dibutyl phthalate (10); in petrolatum	0/14/18	36.0	43.0	16.2
42. Dichlorophene (10); in petrolatum	0/9/10	38.0	40.2	5.47
43. Diethyl phthalate (10); petrolatum	0/5/5	40.2	20.2	0.0
44. Dimethyl phthalate (10); copper oleate (45); in ether	0/9/9	23.4	29.4	20.0
45. Dimethyl phthalate (10); in petrolatum	0/20/20	41.4	43.2	4.1
46. Dimethyl phthalate (5); Indalone (5); olive oil (5); in petrolatum	0/9/10	37.1	44.1	29.4
47. Diphenyl phthalate (10); olive oil (10); in petrolatum	0/6/10	39.3	21.7	0.0
48. Ethyl vanillate (5); in wool fat	0/8/9	22.3	37.6	43.0
49. Hexachlorophene (5); in Prantal	14/42/55	10.4	35.0	70.3
50. Hexachlorophene (10); copper oleate (45); in ether	1/1/9	0.0	35.3	100 (too toxic)
51. Hyamine (5); olive oil (5); in petrolatum	0/9/10	54.3	41.7	0.0
52. Indalone (10); olive oil (10); in petrolatum	0/7/10	34.5	30.5	0.0
53. Kerodex 51 (−)	0/26/28	30.6	37.1	17.0
54. Kolpix A (−)	1/21/22	45.6	45.7	0.0
55. Kolpix D (−)	0/18/21	54.4	50.8	0.0
56. Lanolin (Squibb) (−)	0/6/10	39.0	44.0	11.3
57. N-butyl acetanilide (5); olive oil (5); in petrolatum	0/9/10	57.7	40.2	0.0
58. N-butyl acetanilide in Isopropanol (5); olive oil (5); in petrolatum	0/7/10	69.4	44.1	0.0
59. N-propyl vanillate (3); olive oil (5); in petrolatum	0/9/10	50.8	33.1	0.0
60. Orthogynol (−)	0/8/10	34.5	48.1	28.2
61. Ply-9 Gel (−)	0/11/23	51.2	48.8	0.0
62. Ply-3 Gel (−)	0/9/21	57.2	47.5	0.0
63. Polybor (10); olive oil (10); in petrolatum	0/15/20	39.9	26.1	0.0
64. Prantal (−)	0/17/20	42.8	57.5	25.5
65. Preceptin (−)	1/9/10	24.7	37.6	34.3
66. Pro-derna (−)	0/18/19	49.4	60.5	18.1
67. Protex (2.5); in petrolatum	0/3/4	36.6	21.7	0.0
68. Protex (0.5); olive oil (9.5); in petrolatum	0/11/11	23.1	44.1	47.6

TABLE 3—*Continued*

Ointment and per cent active ingredient	Ratio of mice "protected" (at autopsy)	Average number of worms per mouse (at autopsy)		Per cent relative protection
		"Protected" mice	Control mice	
69. Rotenone resin (30); in acetone*	—	—	—	—
70. Rotenone ointment (0.5) (−)	0/8/10	33.4	44.0	24.0
71. Rotenone ointment (0.25) (−)	0/3/10	98.0	42.0	0.0
72. Rutgers 612 (5); Indalone (5); olive oil (10); in petrolatum	0/8/10	53.1	48.1	0.0
73. Santobrite (0.5); olive oil (10); in petrolatum	0/7/10	49.5	21.7	0.0
74. Silicote (−)	0/19/20	32.4	44.3	26.0
75. Santocel (10); dimethyl phthalate (50); Indalone (16.7); Rutgers 612 (16.6); benzyl benzoate (16.6)	3/27/33	25.0	39.6	37.7
76. Spergon (5); olive oil (5); in petrolatum	0/6/9	7.8	27.8	71.9
77. Spergon (5); in Preceptin	1/8/10	14.7	26.1	43.6
78. Sulphenone (5); olive oil (5); petrolatum	0/7/10	30.1	20.2	0.0
79. Thanite (1); in mineral oil and petrolatum	0/8/10	28.5	40.2	29.1
80. Thanite (5); in wool fat	23/53/60	5.4	39.4	86.0
81. Trio (5); in wool fat	0/8/10	22.5	29.6	30.8
82. West #311 (−)	0/19/20	36.3	34.8	0.0
83. Wool fat (−)	0/20/20	21.6	18.8	0.0
84. Wool fat (90); Santocel (10)	0/8/10	23.1	18.8	0.0
85. Yucca leaf powder (10); olive oil (10); in petrolatum	0/8/10	45.1	33.1	0.0
86. ZAC (−)	2/17/28	17.5	8.3	0.0
87. 555 (dibutyl phthalate (5); Rutgers 612 (5); Indalone (5); olive oil (5); paraffin (20); in petrolatum	0/8/10	32.1	30.5	0.0
88. 786 C (3.33); in petrolatum	0/9/10	74.8	46.8	0.0
89. 786 C (10); in wool fat	1/10/10	32.4	50.6	57.7
90. 640 C (3.33); olive oil (5); in petrolatum	0/7/10	59.1	46.8	0.0
91. 640 C (10); in wool fat	0/9/10	42.7	50.6	15.6
92. 642 C (3.33); olive oil (5); in petrolatum	0/7/10	42.2	46.8	1.0
93. 642 C (10); in wool fat	0/7/10	18.5	50.6	63.2
94. 622 (45); olive oil (2.5); in petrolatum	0/6/10	61.0	30.5	0.0

action of ointments or to the frequent handling of the mice by the neck was not determined. The results of these toxicity tests are summarized in Table 5.

DISCUSSION

General discussion of the ointments. Eighteen compounds yielded excellent results (Table 1). These ointments provided over 99 PRP after the four hour immersion tests. Usually ointments were tested at least twice, not only to secure a larger number of test mice, but also to determine the effectiveness of ointments following storage for varying intervals at room temperature.

Individual ointments, with only one active ingredient, as well as "shotgun"

TABLE 4

Results of in vitro *tests on ointments affording 99–100 per cent relative protection against* Schistosoma mansoni

Ointment and per cent active ingredient	Sample number	Immediate contact*	Delayed contact*	Four-hour diffusion*	72-hour diffusion
1. Actamer (10); Silicote	1	20/15	20/30	20/15	20/15
	2	20/15	20/30	20/15	20/15
2. Actamer (5); Hexachlorophene (3); benzyl benzoate (20); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/30	20/15
3. Benzyl benzoate (20); Covicone with repellents	1	20/15	20/15	20/15	20/15
	2	20/30	20/15	20/15	20/15
4. Hexachlorophene (5); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
5. Hexachlorophene (5); benzyl benzoate (20); copper stearate and copper oleate in ether	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
6. Hexachlorophene (5); copper stearate (40); copper oleate in ether	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
7. Hexachlorophene (3); Covicone with repellents	1	20/15	20/15	20/30	20/15
	2	20/15	20/15	20/30	20/30
8. Hexachlorophene (10); olive oil (10); petrolatum	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
9. Hexachlorophene (10); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
10. Hexachlorophene (3); Pro-derna	1	20/15	12/120	20/15	20/15
	2	20/15	20/45	20/15	20/15
11. Actamer (5); olive oil (5); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
12. Actamer (5); Pro-derna	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
13. Actamer (5); n-butyl acetanilide (5); dimethyl phthalate (5); Indalone (5); wool fat	1	20/15	20/15	20/15	20/30
	2	20/15	20/15	20/15	20/60
14. Benzyl benzoate (20); dibutyl phthalate (10); wool fat	1	20/15	—	20/30	20/120
	2	20/15	20/15	20/15	20/120
15. Captan (5); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
16. Benzyl benzoate (20); Hexachlorophene (10); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
17. Hexachlorophene (1); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
18. Benzyl benzoate (10); Indalone (5); dibutyl phthalate (5); dimethyl phthalate (5); wool fat	1	20/15	0/120	20/15	20/30
	2	20/15	5/120	20/15	20/30
19. Benzyl benzoate (20); wool fat	1	20/15	20/15	20/15	20/15
	2	20/15	20/15	20/15	20/15
20. Copper stearate (20); wool fat	1	0/120	—	—	—
	2	0/120	—	—	—

See footnotes, Table 1.

* Each test was run with 20 cercariae and observed at 15, 30, 45, 60, and 120 minute intervals. Results are shown as the number of dead cercariae divided by observation time.

TABLE 4—*Continued*

Ointment and per cent active ingredient	Sample number	Immediate contact*	Delayed contact*	Four-hour diffusion*	72-hour diffusion
21. Dibutyl phthalate (5); petrolatum	1	20/60	18/120	15/120	20/45
	2	20/45	18/120	12/120	20/45
22. Dimethyl phthalate (5); petrolatum	1	20/60	5/120	5/120	20/60
	2	20/45	3/120	11/120	20/30
23. Indalone (5); petrolatum	1	20/45	20/15	20/15	20/120
	2	20/45	20/15	20/15	20/120
24. Wool fat, anhydrous	1	0/15	—	—	—
	2	0/15	—	—	—
25. N-butyl acetanilide (5); petrolatum	1	17/120	20/15	20/15	19/120
	2	20/45	20/15	20/15	20/30
26. Olive oil	1	0/15	—	—	—
	2	0/15	—	—	—
27. Petrolatum	1	0/15	—	—	—
	2	0/15	—	—	—
28. Pro-derna	1	20/15	0/120	20/15	15/120
	2	20/15	3/120	20/120	20/120
29. Silicote	1	20/15	3/120	20/15	12/120
	2	20/15	0/120	20/30	19/120
30. Controls	1	2/120	—	—	—
	2	5/120	—	—	—
31. Controls	1	0/120	—	—	—
	2	0/120	—	—	—
32. Controls	1	0/120	—	—	—
	2	0/120	—	—	—
33. Controls	1	0/120	—	—	—
	2	—	—	—	—
34. Controls	1	4/120	—	—	—
	2	0/120	—	—	—
35. Controls	1	—	—	—	—
	2	0/120	—	—	—
36. Controls	1	4/120	—	—	—
	2	0/120	—	—	—
37. Controls	1	0/120	—	—	—
	2	4/120	—	—	—
38. Controls	1	1/120	—	—	—
	2	0/120	—	—	—
39. Controls	1	20/15	—	—	—
	2	0/120	—	—	—
40. Controls	1	0/120	—	—	—
	2	0/120	—	—	—

ointments, which included a number of insect repellents, proved to be effective. The remaining ointments tested contained fungicides and bactericides. Actamer (a bactericide) was highly effective, as was hexachlorophene (another bactericidal agent) when mixed with several different vehicles. Captan (an agricultural fungicide) proved to be highly cercaricidal while benzyl benzoate (an insect repellent) also was effective when it was mixed with other repellents and a wool fat base.

TABLE 5

Summary of results of 15 applications of ointments on the survival of white mice during a three-week period

Ointment and per cent active ingredient	Number of mice at start of experiment		Number of mice surviving at three weeks		Per cent surviving	
	Test	Control	Test	Control	Test	Control
1. Actamer (5); Hexachlorophene (3); benzyl benzoate (20); in wool fat	20	29	18	29	90	100
2. Actamer (5); n-butyl acetanilide (5); dimethyl phthalate (5); Indalone (5); and wool fat	20	30	17	28	85	93.3
3. Actamer (5); olive oil (5); and wool fat	20	30	18	28	90	93.3
4. Actamer (5); in Pro-derna	20	30	16	30	80	100
5. Actamer (10); in Silicote	20	30	19	30	95	100
6. Hexachlorophene (5); benzyl benzoate (20); copper stearate (35); copper oleate in ether	20	30	18	30	90	100
7. Benzyl benzoate (20); Covicone with repellents	20	30	18	27	90	90
8. Benzyl benzoate (20); dibutyl phthalate (10); in wool fat	20	30	17	27	85	90
9. Captan (5); in wool fat	20	30	17	28	85	93.3
10. Hexachlorophene (5); copper stearate (40); copper oleate (55)	20	29	2	29	10	100
11. Hexachlorophene (3); and Covicone with repellents	20	29	18	29	90	100
12. Hexachlorophene (3); in Pro-derna	20	29	19	29	95	100
13. Hexachlorophene (5); in wool fat	20	29	18	29	90	100
14. Hexachlorophene (1); in wool fat	20	30	18	27	90	90

See footnote, Table 1.

Fifteen compounds were tested which provided 90 to 99 PRP against the penetration of cercariae of *S. mansoni* (Table 2). In most instances these compounds gave complete protection on the initial four-hour immersion tests, but failed to furnish it when tested further. Spergon (an insecticide), copper-8-quinolinolate, and copper cunilate initially showed promise, but all three failed subsequently to give complete protection.

Benzyl benzoate when mixed with other repellents and in a pertinaceous base gave 100 per cent protection, while aqueous solutions yielded less (Table 1). Actamer (a bactericide) provided less than 99 PRP in petrolatum, while with Silicote, Pro-derna, and wool fat it gave complete protection.

Ninety-four compounds furnished less than 90 PRP in the four-hour immersion tests (Table 3). Since time was short further tests on these compounds were deemed inadvisable. Most of these were ointments giving 90 to 100 PRP in the preliminary screening tests. This screening procedure was recognized as not being definitive because the ointment-protected surface of the mouse was immersed in a small quantity of water which contained the cercariae. Manifestly, the diffusion of some test materials into the water could reach a concentration

sufficient to kill the carcariae, thus producing a false positive test. However, it was believed that sufficient information could be gathered from this test to separate the most promising chemicals from the less effective ones. On this basis all compounds giving less than 90 PRP were discarded.

Four-hour immersion tests on Schistosoma mansoni. The object of the four-hour immersion tests was to set up a more critical test which would give further information on ointments which offered complete protection in the preliminary screening tests. They were also instituted to simulate, as nearly as possible, the "washing action" a person would encounter working in water under field conditions. It is believed that such a demanding test would effectively eliminate those preparations which would not stand up under field conditions. A continuous flow of water through the aquaria was instituted to prevent an accumulation of any agent which might wash off the mouse. In this way, any chemical which diffused out of the ointment was removed from the aquaria. This prevented any possible accumulation in the water of materials toxic to the cercariae. The unrestrained mice had ample opportunity to scratch, wash, lick off, or brush the ointment from their bellies during the four hour test.

Controls. The control mice were processed and exposed in the same manner as the test mice except that they did not receive any ointment. In each experiment, control mice were exposed under the same conditions and were infected from the same pool of cercariae as the test mice. At autopsy, the total number of *S. mansoni* adults found were averaged, and the per cent relative protection computed. The whole series of experiments (121 groups of controls), averaged 37.8 *S. mansoni* adults per mouse, ranging from an average of 8.8 worms per mouse to 56.7 worms per mouse. Such variations were usually fairly constant for a particular experiment and would either all be high or low. This may have represented variations in the viability of cercariae affected by biological or physical factors.

Four-hour immersion tests on Schistosoma japonicum. A small shipment of infected *Oncomelania formosana* and *O. nosophora*, two of the snail hosts for *Schistosoma japonicum*, was made available. As these snails were received in early summer, and since time was short only in vivo tests were carried out. Three series of four-hour immersion tests were run involving three of the most promising ointments. Unfortunately, the results of these experiments on *S. japonicum* were inconclusive since insufficient numbers of cercariae were obtained to produce a significant infection in the controls.

The infection site. From comparative histological studies of skin from the mouse belly, neck and tail, with human skin from the forearm and leg, it was apparent that the abdominal surface of the mouse more nearly approximated the human skin than such other areas as the tail and neck which were used by other investigators (Wright *et al.*, 1948; Ferguson *et al.*, 1946; Hunter *et al.*, 1952a; Bauman *et al.*, 1954, 1955). Comparisons based upon photomicrographs of the tail and belly skin of the mouse clearly show the latter to be thinner with a more delicate *stratum corneum*, a condition much more comparable to man (Figs. 3, 4, and 5). As shown by Standen (1953), human skin from the area most apt to be exposed,

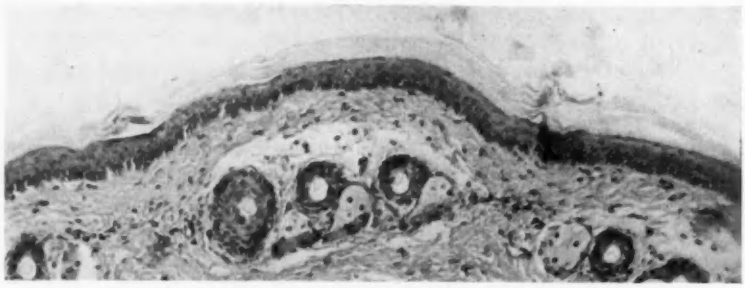

FIG. 3. Section of tail of mouse showing thickened *stratum corneum*. (H & E stain. ×100.)

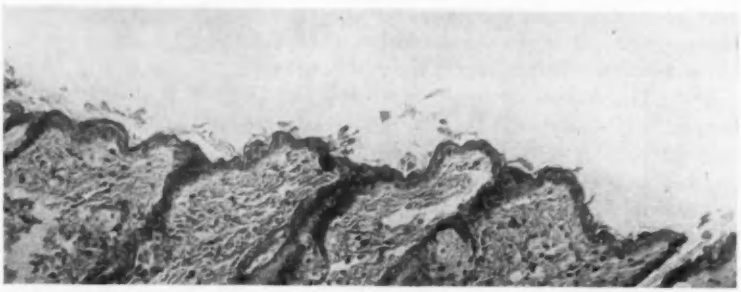

FIG. 4. Section of belly skin of mouse showing thin *stratum corneum*. (H & E stain. ×100.)

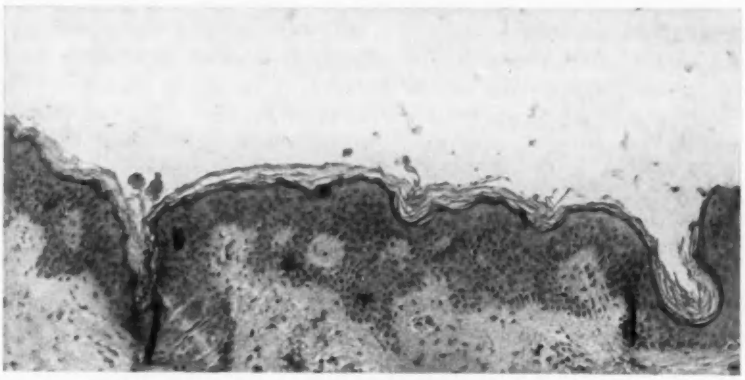

FIG. 5. Section of human skin from the leg. Compare with Figs. 3 and 4. (H & E stain. ×100.)

i.e., the arm and leg, usually possessed a thicker *stratum corneum* than occurs in mouse belly skin. Gordon and Griffiths (1951) using *S. mansoni*, and Pan (personal communication) with *S. japonicum*, have shown that schistosome cercariae do not usually use the hair follicles in penetrating the skin. It was felt, therefore, that if an ointment protects the skin of a host, such as a mouse, against penetrating schistosome cercariae, it should prove to be even more efficient when applied to the thicker skin of man. If this is so, it follows that the belly skin of the mouse, being thinner than the other two sites mentioned, furnishes the least protection, per se, and hence is the best test site for protection experiments.

In vitro tests. In vitro tests were conducted to determine the mode of action of the effective ointments and their constituent parts (Table 5). In vitro tests were designated as "immediate contact tests," "delayed contact tests," "four-hour diffusion tests, and "72-hour diffusion tests."

Properties of an ideal protective ointment. The properties of an ideal protective ointment have been listed by Wright *et al.* (1948); Hunter *et al.* (1952a), and others. Expanded criteria should include:

1. Protection to man against *Schistosoma japonicum*, *S. haematobium*, and *S. mansoni* for a minimum period of four hours.
2. Non-toxicity for man in the concentration used, either as a primary irritant or cumulative poison.
3. Physical and chemical stability during transportation and storage in all extremes of weather.
4. Ease of application and removal.
5. Aesthetic acceptability.
6. Ready availability at low cost.
7. Possible repellent properties for noxious insects, ticks, mites, leeches, etc.

Semi-qualitative toxicity tests. In order to secure further data on the most effective protective ointments, semi-qualitative toxicity tests were run on a number of the best ointments. Fifteen applications, covering a three-week period, were applied to the shaved belly of 20 mice. Thirty mice were used for controls. These results are summarized in Table 5.

The ingredients of the best ointments. The 16 ingredients which were used in the formulation of the ointments providing 99 to 100 PRP included: Actamer, benzyl benzoate, Captan, copper oleate, copper stearate, Covicone with repellents, dibutyl phthalate, dimethyl phthalate, hexachlorophene, Indalone, n-butyl acetanilide, olive oil, petrolatum, Proderna, Silicote, and wool fat. Data on these are summarized individually (*vide infra*) and information on the ointments themselves are compiled in Table 1.

Actamer (2,2′ thiobis (4,6-dichlorophenol)) is the trade name for bithionol, the generic name accepted by the Council on Chemistry and Pharmacy of the American Medical Association. It is a white crystalline solid relatively insoluble in water and is chemically and physically stable for three years at 50°C. (Anon. 1952, 1953). Actamer is an efficient bacteriostatic agent often used in soaps.

Patch tests on 200 male and female subjects indicated that Actamer (2%) in a soap base was not a primary irritant or skin sensitizer (Anon., 1953). Soap containing 2% Actamer was fed to white rats. Control animals received soap alone. Since the deaths in both groups were essentially the same it appears probable that the soap base and not the Actamer was responsible for the losses. Dogs administered doses of 3.2, 4.7, and 7.0 grams of Actamer per kilo of body weight survived, showing no signs of illness and displaying normal appetite and activity (Anon., 1953).

Actamer, as a 1% solution in dimethyl phthalate, was subjected to percutaneous toxicity studies over a test period of 90 days. The solutions were applied daily, five days per week, to the shaved skin of three groups of rabbits at respective dosage levels of 10 mg., 20 mg., and 40 mg. per kilo of body weight. At the end of 90 days, five animals from each group were sacrificed and histological examinations of several organs were made. No pathological changes were noted which could be attributed to Actamer (Anon., 1953).

The ophthalmic toxicity studies were made on rabbits and the effects of Actamer alone and in four different media were observed. The results indicated that undiluted Actamer as a dry powder produced severe conjunctivitis with injection of the corneal vessels within four hours. These symptoms diminished during the next four hours. Actamer, used in soap media, produced little, if any, irritation (Anon., 1953).

In the present study, Actamer was incorporated into ten different ointments, utilizing seven different vehicles. The PRP of these ten ointments ranged from 94.2 to 100 (Tables 1, 2).

Benzyl benzoate is a water-white fluid, insoluble in water. It is used as a repellent for fleas, mites, and chiggers. The Merck Index (1940) lists benzyl benzoate as an anti-spasmodic, and it is used commercially in confections and flavoring for chewing gum. Brown (1951) claims that benzyl benzoate may cause a dermatitis in some children.

In the present study, benzyl benzoate was incorporated into 14 different compounds utilizing nine different bases. The PRP ranged from 10 to 100 per cent. In vitro tests of benzyl benzoate showed that the active agent remained in the ointment in sufficient quantity to kill, while cercaricidal amounts diffused into the water to kill cercariae in 15 minutes.

Captan is the trade name of the California Spray-Chemical Corporation for n-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide. It is a white crystalline solid used as an agricultural fungicide. The oral toxicity of Captan is on the order of 9 mg. technical Captan per kilogram of body weight in rats. At the 10–15 gm./kg. levels, diarrhea and weight loss were observed shortly after the dose. Extensive pathological examination of the animals showed no lesions that could be attributed to this compound. It appears probable that the low oral toxicity results from inefficient absorption of the chemical from the gastrointestinal tract or the rapid detoxification in the body and elimination as water-soluble products. This would explain the failure to find Captan in the tissues and feces of animals which have been fed large amounts (Hansen *et al.*, 1954). Chronic

toxicity was tested by feeding rats a diet in which 0.025%, 0.25%, and 1.0% Captan was incorporated over a period of two years. No gross or microscopic pathologic changes could be found which might be definitely attributable to Captan in the diet (Batte, E. G., personal communication).

In the present cercaricidal study, Captan was incorporated into three different ointments utilizing three bases: wool fat which gave 99.8 PRP; petralotum and Prantal produced only 35 and 39.8 PRP, respectively.

Copper oleate is a dark blue, semi-solid, viscous substance which is insoluble in water and in ethyl alcohol, but freely soluble in ether and, to some extent, in other fat solvents.

Copper oleate in ether gave 98.4 PRP in the preliminary screening tests. Previously similar experiments by Moon and Hunter (1954, 1955) gave 86.8 to 97.1 PRP to white mice against the penetration of *S. mansoni* cercariae. The copper oleate was tested in both ether and petrolatum. It is interesting to note that in no instance was complete protection secured against *S. mansoni* cercariae although copper oleate did protect white mice completely against the penetration of cercariae of *S. japonicum* (Kaufman, Hunter, and Pan, 1949; Hunter, Kaufman, and Pan, 1952a). Copper oleate also protected paddy workers for six hours against dermatitis-producing bird schistosomes cercariae (Hunter *et al.*, 1950; Hunter *et al.*, 1952b). It is believed that some physiologic difference exists between the cercariae of *S. mansoni* and *S. japonicum* which accounts for this difference in protection afforded white mice. This seems to be the only logical interpretation as the same techniques as well as some of the same workers participated in both studies.

Hunter *et al.* (1952a) ran semi-qualitative toxicity tests on white mice by smearing them with copper oleate every third day for 18–23 applications. No evidence of toxicity was found when these were compared with the controls. Similar tests were not made during this present study as a summary of information on the toxicity of copper oleate was furnished through the Armed Forces Epidemiological Board and the Army Chemical Center.

Copper stearate is a blue, finely-powdered solid. Toxicity tests were not run by this laboratory on this compound, nor have any toxicological data been received. Turner (1950), however, states that copper stearate is poisonous. This compound proved to be only 45.8 per cent effective, in the preliminary screening test. Copper stearate failed to kill cercariae at the end of 120 minutes in the immediate contact test, due probably to its insolubility in water.

Covicone is a commercially prepared skin protectant cream. It is a plasticized combination of silicone (dimethyl polysiloxane), nitrocellulose and castor oil suspended in a greaseless vanishing cream base. Several insect repellents have been added to Covicone such as "6-2-2" (E-4856), and M-2020 (E-5420), both U. S. Army repellents. Both Covicone alone as well as the repellents that were added have been tested for toxicity by the government and other agencies and were found to be relatively non-toxic in low dilutions.

Covicone Repellent Cream (i.e., E-4856) with 6-2-2 repellent added, gave complete protection in the preliminary screening tests, but only 53 PRP in

the four hour immersion tests. The loss of experimental and control mice was approximately identical. In spite of the difference in the methods used, our results correspond closely to those obtained by Bauman *et al.* (1954, 1955). Bauman showed that Covicone Repellent Cream (E-4856) gave 100 per cent protection to mice as long as six hours after application of the ointment to the tails of mice. During this period the mice were restrained, and had no contact with water until their tails were exposed to cercariae-infested water for 30–60 minute test period. In tests where the tails were allowed to soak in water, the protective agent evidently washed out as they only obtained a low degree of protection. These findings in general confirmed the results secured in this laboratory with the four hour immersion test.

Dibutyl phthalate is an acaricide and mosquito repellent. Toxicity tests on this compound itself were not run as the available data in the literature indicated this was non-toxic (Turner, 1950; Mackie *et al.*, 1954). Dibutyl phthalate in petrolatum proved 99.9 per cent efficacious in the preliminary screening tests. The test mice had a mortality of 45 per cent in contrast to only 20 per cent in the controls. The four hour immersion test yielded only 16.2 PRP with a mortality of 22 per cent for the test mice and none for the controls. The relatively high loss among the mice "protected" with dibutyl phthalate indicates that this substance was toxic for mice in these concentrations. The in vitro tests showed the compound to be relatively ineffective.

Dimethyl phthalate is a widely used repellent. Turner (1950) and Shepard (1951) say that dimethyl phthalate is not a marked irritant except to the eyes. Dimethyl phthalate furnished complete protection in the preliminary screening tests; there was a 17 and 22 per cent loss in the test and control mice, respectively. However, this chemical failed in the four hour immersion test since it provided only 4.1 PRP. Dimethyl phthalate was relatively ineffective as a cercaricidal agent in the in vitro tests.

Hexachlorophene is the generic name for "G-11," an antiseptic and germicide. Hexachlorophene is a chlorinated bisphenol, and can be chemically characterized as 2,2′-methylene bis (3,4,6-trichlorophenol). Data on the acute oral toxicity of hexachlorophene varies. Nickerson (personal communication) found an LD_{50} of 187 mg./kg. for mice with a range of 166 to 209 mg./kg., whereas Florestano (1949) reported an LD_{50} of only 80 mg./kg. Further studies over a 30-day period indicated that hexachlorophene is mildly toxic when fed to rats in a concentration of 1:5,000 and more definitely toxic when fed in a concentration of 1:2,500. Traub *et al.* (1944) performed patch tests on more than 600 patients using 0.5 per cent and one per cent hexachlorophene in petrolatum; all tests were negative. These results indicated that hexachlorophene was usually non-irritating and non-sensitizing to the skin of man when used in these concentrations. Furthermore, a single instillation into the conjunctival sac of the rabbit's eye of 0.1 ml. of solution of two per cent hexachlorophene is not irritating.

In the current experiments hexachlorophene was incorporated into ten different ointments utilizing seven different ointment bases, the PRP of these compounds ranging from 70.3 to 100 (Tables 1, 2, 3). In all in vitro experiments, hexachlorophene was cercaricidal within 15 minutes.

Indalone is the trade name for n-butyl mesityl oxide oxalate. It has been used for several years as a mosquito repellent. In view of this it was assumed that its toxic properties had been investigated and found to be relatively low for man in the concentrations normally used.

Ten per cent Indalone in petrolatum proved to be 100 per cent effective in the preliminary screening tests; the loss in the experimental and control mice was negligible in both groups. However, following the four hour immersion test, Indalone-treated mice had as many or more adult worms at autopsy than the control mice. Apparently Indalone per se was fairly toxic as thirty per cent of the test mice died compared to only nine per cent of the controls. In vitro tests on Indalone were inconclusive (Table 4).

N-butyl acetanilide is used as a repellent in clothing for ticks, chiggers, and mosquitoes. However, this compound is highly toxic to experimental animals (mice) and to man when used in too concentrated a form. As one of the ingredients of M-1960, it has not been above suspicion when impregnated into uniforms. Further data should be available through the Army Chemical Center.

The preliminary screening test with n-butyl acetanilide gave complete protection. However, the four hour immersion test failed completely, as the chemical gave virtually no protection. Twenty per cent of the test animals died compared with 32 per cent of the controls. N-butyl acetanilide was relatively ineffective when subjected to in vitro tests (Table 4).

Pro-derna is a commercially prepared skin protectant containing 52.5 per cent silicone (polydimethylsiloxane) in an inert, hydrated aluminum base. Suskind (1954), after tests involving 114 subjects in one experiment, states that the silicone formulations have a very low capacity for inducing irritation and sensitization of human skin.

Pro-derna provided 96 PRP in the preliminary screening tests; in these tests 14 per cent of the test mice and 40 per cent of the controls died. In the four hour immersion test, Pro-derna protected only 18.1 per cent of the test mice; however, there was only a four per cent mortality in the test mice in contrast to 20 per cent in the controls. In the in vitro tests Pro-derna killed all cercariae in 15 minutes in the immediate contact tests, but proved ineffective in the remaining in vitro experiments.

Silicote is composed of 30 per cent silicone oils in a petrolatum base. It is used as a skin protectant cream. Talbot *et al.* (1951) stated that toxicological studies have shown that the silicones have little or no untoward reactions when administered by mouth (up to two per cent of body weight), inhaled (as high as 4,000 parts per million), or applied to the skin.

Silicote killed all cercariae within 15 minutes of immediate contact but proved relatively ineffective in the other in vitro tests (Table 4). Silicote alone gave 90.8 PRP in the preliminary screening test. In these tests only twelve per cent of the test mice died compared with 30 per cent of the controls. In the four hour immersion test, Silicote was 26 per cent effective with five per cent mortality in the test mice and none in the controls.

Olive oil is not toxic. It was used as a levigating agent. The preliminary screening tests indicate that olive oil offered no protection whatsoever to the penetra-

tion of *S. mansoni* cercariae. In vitro tests were discontinued after immediate contact tests, since no cercariae were killed.

Petrolatum. In the preliminary screening tests, petrolatum afforded 29.4 PRP; six per cent of the test mice died compared with a third of the controls. This substance was non-toxic. Petrolatum was used as a base in nearly all of the preliminary screening tests, but in the four hour immersion tests, wool fat was substituted as it proved to be a more satisfactory base. It is worthy of noting that both hexachlorophene and Actamer when mixed with petrolatum gave respectively 100 and 96 PRP. Yet petrolatum failed to kill cercariae in the in vitro tests.

Wool fat (*adeps lanae*, U.S.P., anhydrous) is obtained from the wool of sheep. It was tested alone in the four hour immersion test and provided no protection to mice against the penetration of the cercariae of *S. mansoni*. Incidentally, there was no mortality in either the control or test mice. Wool fat did not kill the cercariae in the in vitro tests.

The best ointments in the four hour immersion test. Eighteen ointments withstood the four hour immersion test and proved 99 to 100 per cent effective in protecting white mice against infection by the cercariae of *S. mansoni* (Table 1). All of the first 10 ointments listed gave complete protection; these are arranged alphabetically. The eight remaining ointments are listed in order of their efficiency. The 18 ointments follow: Actamer (10%) in Silicote; Actamer (5%), Hexachlorophene (3%), benzyl benzoate (20%) in anhydrous wool fat; benzyl benzoate (20%) in Covicone with added repellents (E-4856); Hexachlorophene (10%) in wool fat; Hexachlorophene (5%) in wool fat; Hexachlorophene (5%), benzyl benzoate (20%), copper stearate (40%) in copper oleate and ether; Hexachlorophene (5%), copper stearate (40%) in copper oleate and ether; Hexachlorophene (3%) in Covicone with repellents added (E-4856); Hexachlorophene (10%), olive oil (10%) in petrolatum; Hexachlorophene (3%) in Pro-derna; Actamer (5%), olive oil (5%) in wool fat; Actamer (5%) in Proderna; Actamer (5%), n-butyl acetanilide (5%), dimethyl phthalate (5%), Indalone (5%) in wool fat; benzyl benzoate (20%), dibutyl phthalate (10%) in wool fat; Captan (5%) in wool fat; benzyl benzoate (20%), Hexachlorophene (10%) in wool fat; Hexachlorophene (1%) in wool fat; and benzyl benzoate (10%), Indalone (5%), dibutyl phthalate (5%), dimethyl phthalate (5%) in wool fat. The results of these tests are summarized in Table 1.

Recommended ointments. The 18 ointments giving 99–100 PRP have been discussed (*vide supra*). It remains to select a few of the best and suggest that these compounds be considered first. To facilitate making such a selection a table has been prepared listing the more important properties of an ideal protective ointment (Table 6). In addition to the factors listed in the table, the per cent of mice surviving the tests in comparison with the controls was also considered. Obviously such a selection is affected by a variety of unknown, or only conjectured factors, such as the summer heat. No consideration was given to manufacturing costs as these items are unknown. Keeping these various factors in mind, the following are recommended as probably the best:

1. Ointment # 1—Actamer (10%) and Silicote;

TABLE 6

Summary of the 18 best ointments compared with the characteristics of the ideal protective ointment

Ointment	Give 100% protection to mice against *S. Mansoni*	Toxic to mice	Stable during experiments	Easy to apply	Easy to remove	Stain clothing	Cost	Repellent properties
1. Actamer (10%); Silicote	Yes	No	Yes	Yes	Fairly	No	?	No
2. Actamer (5%); Hexachlorophene (3%); benzyl benzoate (20%) wool fat	Yes	No	Yes	Yes	Fairly	Temp.	?	Probably
3. Benzyl benzoate (20%); Covicone with repellents	Yes	No	No	No	Fairly	Temp.	?	Probably
4. Hexachlorophene (10%); wool fat	Yes	Yes	Yes	Yes	Fairly	Temp.	?	No
5. Hexachlorophene (5%); wool fat	Yes	No	Yes	Yes	Fairly	Temp.	?	No
6. Hexachlorophene (5); benzyl benzoate (20%); copper stearate and copper oleate in ether	Yes	No	No	No	No	Yes	?	Probably
7. Hexachlorophene (5%); copper stearate (40%); copper oleate in ether	Yes	Yes	No	No	No	Yes	?	No
8. Hexachlorophene (3%); Covicone with repellents	Yes	No	No	No	Fairly	Temp.	?	Probably
9. Hexachlorophene (10%); olive oil (10%); petrolatum	Yes	No	Yes	Yes	Fairly	Temp.	?	No
10. Hexachlorophene (3%); Pro-derna	Yes	No	Yes	Yes	Yes	No	?	No
11. Actamer (5%); olive oil (5%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	No
12. Actamer (5%); Pro-derna	No	No	Yes	Yes	Yes	No	?	No
13. Actamer (5%); n-butyl acetanilide (5%); dimethyl phthalate (5%); Indalone (5%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	Probably
14. Benzyl benzoate (20%); dibutyl phthalate (10%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	Probably
15. Captan (5%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	No
16. Benzyl benzoate (20%); Hexachlorophene (10%); wool fat	No	Yes	Yes	Yes	Fairly	Temp.	?	Probably
17. Hexachlorophene (1%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	No
18. Benzyl benzoate (10%); Indalone (5%); dibutyl phthalate (5%); dimethyl phthalate (5%); wool fat	No	No	Yes	Yes	Fairly	Temp.	?	Probably

2. Ointment #2—Actamer (5%), Hexachlorophene (3%), benzyl benzoate (20%) and wool fat;
3. Ointment #3—Benzyl benzoate (20%) and Covicone with repellents;
4. Ointment #5—Hexachlorophene (5%) and wool fat;
5. Ointment #10—Hexachlorophene (3%) and Pro-derna.

It must be realized that the selection of these five ointments was not easy, because all eighteen gave over 99 PRP. In addition some of the last eight hold considerable promise and probably failed due to some undetected accident such as dropping one or two cercariae from a pipette onto the unprotected skin of a mouse. In view of this it appears probable that others such as Actamer (5%) and olive oil (5%) in wool fat; Actamer (5%) and Pro-derna; and Captan (5%) in wool fat should also be considered for further testing as only one or two adults were recovered from a single "protected" mouse.

It is recommended that those mentioned above be analyzed by the Army Chemical Center for toxicity to man and that they then be tested on laboratory animals against the cercariae of both *S. haematobium* and *S. japonicum*. Those giving protection should then be further tested against the cercariae of dermatitis-producing bird schistosomes using sensitized individuals as test "animals". Such experiments should then be followed by field tests of man using all three human species of schistosomes.

SUMMARY

During the second year of screening potential protective ointments against schistosomiasis using white mice and cercariae of *Schistosoma mansoni*, 127 compounds were tested with the following results:

Ten ointments gave complete protection following the "four hour immersion test." These were: Actamer (10%) in Silicote; Actamer (5%), Hexachlorophene (3%) and benzyl benzoate (20%) in wool fat; benzyl benzoate (20%) and Abbott's Covicone with repellents (E-4856); Hexachlorophene (10%) in wool fat; Hexachlorophene (5%) in wool fat; Hexachlorophene (5%), benzyl benzoate (20%), copper stearate and copper oleate in ether; Hexachlorophene (5%), copper stearate (40%), with copper oleate (40%) in ether; Hexathlorophene (3%) and Abbott's Covicone with repellents (E-4856); Hexachlorophene (10%) and olive oil (10%) in petrolatum; and Hexachlorophene (3%) in Pro-derna. Eight more gave over 99 PRP with the four hour immersion test; fifteen provided 90 to 98.9 PRP while 94 gave less than 90. The four hour immersion test with cercariae of *S. japonicum* gave equivocal results due to the small number of cercariae available.

Of the 10 ointments providing complete protection following the four hour immersion test mentioned above, the following were selected as most nearly fulfilling the prerequisites of an ideal protective ointment: Actamer (10%) and Silicote; Actamer (5%), Hexachlorophene (3%) and benzyl benzoate (20%) in wool fat; benzyl benzoate (20%) and Covicone with repellents; Hexachlorophene (5%) and wool fat; and Hexachlorophene (3%) and Pro-derna. These should be checked first for toxicity to man and then tested against other human schistosomes using experimental animals.

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REFERENCES

ANONYMOUS, 1952. Actamer. Monsanto Tech. Bull. 0-38, 1 p.

ANONYMOUS, 1953. Actamer. Monsanto Tech. Bull. ODB-53-12, 21 pp.

BAUMAN, P. M., DURAN, L. B., AND MCMULLEN, D. B., 1954. Effectiveness of Abbott's insect repellent cream (E-4856) as a protective barrier against *Schistosoma mansoni* in mice, *J. Parasit.* (Suppl.) **40**: 35.

———, 1956. Effectiveness of Abbott's insect repellent cream (E-4856) as a barrier against *Schistosoma mansoni* in mice, *J. Parasit.* **41**: 298–301.

BROWN, A. W. A., 1951. *Insect Control by Chemical Means*. vii+, 817 pp. John Wiley and Sons, Inc., N. Y.

FERGUSON, M. S., GRAHAM, O. H., BANG, F. B., AND HAIRSTON, N. G., 1946. Studies on schistosomiasis japonica. V. Protection experiments against schistosomiasis japonica, *Am. J. Hyg.* **44**: 367–378.

FLORESTANO, H. J., 1949. Tuberculocidal activity and toxicity of some diphenylmethane derivatives, *J. Pharm. Exptl. Therap.* **96**: 238–248.

GORDON, R. M., AND GRIFFITHS, R. B., 1951. Observations on the means by which the cercariae of *Schistosoma mansoni* penetrate mammalian skin, together with an account of certain morphological changes observed in the newly penetrated larvae, *Ann. Trop. Med. and Parasit.* **45**: 227–243.

HANSEN, J. W., KOHN, G. K., AND OSPENSEN, J. N., 1954. Research report on Captan decomposition, California Spray-Chemical Corporation. 5 pp.

HUNTER, G., III, RITCHIE, L. S., AND TANABE, H., 1950. The epidemiology of schistosome dermatitis (Koganbyo) in Japan, *J. Parasit.* (Suppl.) **36**: 12.

HUNTER, GEORGE W., III, KAUFMAN, E. H., JR., AND PAN, C., 1952a. Studies on schistosomiasis. III. Copper oleate ointment in protection experiments against *Schistosoma japonicum*, *Exptl. Parasit.* **1**: 168–175.

HUNTER, GEORGE W., III, RITCHIE, L. S., TANABE, H., NAGANO, K., PAN, C., AND YOKOGAWA, M., 1952b. Studies on schistosomiasis. V. Testing protective ointments against schistosomiasis by using schistosome dermatitis producing cercariae, *Trans. Roy. Soc. Trop. Med. and Hyg.* **46**: 201–206.

KAUFMAN, E., HUNTER, G. W., III, AND PAN, C., 1949. Protection experiments with copper oleate ointment against schistosomiasis, *J. Parasit.* (Suppl.) **35**: 29.

KEMP, H. A., HUNTER, G. W., III, WILKINS, O. P., SMALLEY, H. E., AND DASHIELL, M. A., 1954. Studies on schistosomiasis. XI. Some ointments examined for protection against *Schistosoma mansoni* cercariae in preliminary screening tests, *J. Parasit.* (Suppl.) **40**: 34. (also in *Mil. Med.* **118**—in press).

KEMP, HARDY A., AND HUNTER, G. W., III., 1955. Screening of potential protective ointments against schistosomiasis, *Ann. Rept. to the Commission on Parasitic Diseases of the Armed Forces Epidemiological Board*. ii+, 28 pp.

MACKIE, T. T., HUNTER, G. W., III, WORTH, C. B., 1954. *A Manual of Tropical Medicine*. 2nd ed. xxii+ 907 pp. W. B. Saunders Co., Philadelphia.

Merck, Index. 1940. 6th ed. xiv+ 1167 pp. Merck and Company, Inc., Rahway, N. J.

MOON, A. P., AND HUNTER, G. W., III., 1954. Studies on schistosomiasis. VIII. Failure of

copper oleate to protect mice against penetration of the skin by cercariae of *Schistosoma mansoni*, *J. Parasit.* (Suppl.) **40:** 43.

———, 1955. Studies on schistosomiasis. VIII. Failure of copper oleate to protect mice against the penetration of the skin by cercariae of *Schistosoma mansoni*, *Exptl. Parasit.* **4:** 165–171.

PAN, C. AND HUNTER, G. W., III., 1951. A modified perfusion technique for the recovery of schistosomes, *J. Lab. Clin. Med.* **37:** 815–816.

RADKE, M. G., HUNTER, G. W., III, MOON, A. P., POTTS, D. E., AND WILLIAMS, J. S., 1953. Studies on schistosomiasis. IX. Studies on some ointments for protection against *Schistosoma mansoni*, *J. Parasit.* (Suppl.) **39:** 19.

———, 1954. Studies on schistosomiasis. IX. Results of screening some ointments for protection against *Schistosoma mansoni*, *Mil. Surg.* **115:** 401–405.

SHEPARD, HAROLD H., 1951. *The Chemistry and Action of Insecticides*, vii+ 504 pp. McGraw-Hill Book Co., Inc., N. Y.

STANDEN, O. D., 1953. The penetration of the cercariae of *Schistosoma mansoni* into the skin and lymphatics of the mouse, *Trans. Roy. Soc. Trop. Med. Hyg.* **47:** 292–298.

SUSKIND, R. R., 1954. Industrial and laboratory evaluation of a silicone protective cream, *Arch. Ind. Hyg. and Occup. Med.* **9:** 101–112.

TALBOT, J. R., MACGREGOR, J. K., AND CROWE, F. W., 1951. The use of silicote as a skin protectant, *J. Investig. Dermat.* **17:** 125–126.

TRAUB, E. F., NEWHALL, C. A., AND FULLER, J. R., 1944. The value of a new compound used in soap to reduce the bacterial flora of the human skin, *Surg., Gynec., and Obst.* **79:** 205–216.

TURNER, F. M., 1950. *The Condensed Chemical Dictionary*, 4th ed., ix+, 726 pp. Reinhold Publ. Corp., N. Y.

WRIGHT, W. H., BAUMAN, P. M., AND FRY, N., 1948. The control of schistosomiasis japonica. VII. Studies on the value of repellents and repellent ointments as a protection against schistosomiasis japonica, *Am. J. Hyg.* **47:** 45–52.

YOLLES, T. K., MOORE, D. V., DEGUISTI, D. L., RIPSON, C. A., AND MELENEY, H. E., 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes, *J. Parasit.* **33:** 419–426.

THE RECORD OF *ANCYLOSTOMA BRAZILIENSE* AS AN INTESTINAL PARASITE OF MAN IN NORTH AMERICA

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Records of patent *Ancylostoma braziliense* infections in man have been reviewed by Stoll (1947) and Biocca (1951). Biocca's studies indicated that *A. ceylanicum*, generally regarded as a synonym of *A. braziliense*, is a valid species which in some parts of the world is a relatively frequent intestinal parasite of man, whereas the occurrence of *A. braziliense* in the intestine of man will require further proof. *A. braziliense* in a Texas boy, reported by Dove (1928, 1932), was accepted by Stoll as a verified record, and is the only such report of human infection in North America.

Dove's report was based chiefly on the following observations:

(1) Between September, 1927 and February, 1928, larvae cultured from a boy's stool were inoculated into three "clean" kittens and one pup. Worms later removed from these animals were identified as *Ancylostoma braziliense*. These findings were regarded as proof that the boy harbored an intestinal infection of *A. braziliense*. Later, in May, 1928, following inoculations of larvae derived from the same boy, *A. caninum* was recovered from two pups and a kitten. Control animals apparently were not used.

(2) On December 4, 1927, March 24, and again in May, 1928, larvae cultured from the boy's feces were placed on the skin of a volunteer. The first exposure was followed by mild, transient, papular reaction; the second and third produced swelling and short linear lesions that lasted two weeks or less.

(3) Cultures made previous to the third inoculation, at the time of inoculation and during the following two weeks resulted only in failure to recover larvae. Other methods of fecal examination apparently were not used.

(4) On the 23rd day following the third inoculation, hookworm larvae were cultured from a sample of the volunteer's stool and on the 30th day 18 female and 14 male specimens of *Necator americanus* were expelled by treatment. Size and maturity of the worms were not noted.

Dove concluded that "the development of *Necator* in the volunteer and of *A. caninum* in the pups and kitten demonstrated that the boy (D.T.) was infected with these two species of hookworms, and that at this time there was no further infection of *A. braziliense*."

Dove's demonstration is not convincing. It is possible that his experimental pups and kittens were free from natural hookworm infections, but the experiments could have been strengthened by the inclusion of control animals. His methods of culturing feces as a means of establishing presence or absence of hookworm infection possibly were not trustworthy, because in some instances cultures of large quantities of feces produced very scanty yields of larvae. Dove

himself recognized that the skin reactions to the three experimental inoculations were no less characteristic of *Necator* than of *A. braziliense* or *A. caninum*. Finally, it is unlikely that *Necator* would reach maturity in 23 days since carefully observed *Necator* infections have not produced eggs earlier than the end of the 6th or during the 7th week after penetration of the larvae.

The writer's conclusion is that the Texas boy in question probably was infected with *Necator americanus* only, and that there is therefore no verified record of *A. braziliense* in man in North America.

REFERENCES

Biocca, E., 1951. On *Ancylostoma braziliense* (de Faria, 1910) and its morphological differentiation from *A. ceylanicum* (Looss, 1911), *J. Helminthol.* **25**: 1–10.

Dove, W. E., 1928. An intestinal infection of *Ancylostoma braziliense* in a boy and skin lesions produced with larvae from this strain, *J. Parasitol.* **15**: 136–137.

Dove, W. E., 1932. Further studies on *Ancylostoma braziliense* and the etiology of creeping eruption, *Am. J. Hyg.* **15**: 664–711.

Stoll, N. R., 1947. Concerning reports of *Ancylostoma braziliense*, *J. Parasitol.* **33**: 170–171.

EFFECTS OF TETRACHLORETHYLENE WITHOUT A PURGE, FOLLOWED BY FERROUS SULFATE IN ANCYLOSTOMIASIS

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Carr *et al.* (1954), working in the Dominican Republic, showed that, in the treatment of ancylostomiasis, tetrachlorethylene is more effective in removing worms and provokes less complaint on the part of the patients when it is not followed by a saline purgative. Because of the practical importance of such a finding, we were interested in discovering whether the same therapeutic procedure would be equally effective on the African continent. At the same time, we took occasion to observe the effect of ferrous sulfate treatment on hemoglobin regeneration.

MATERIALS AND METHODS

For these purposes, 165 patients with hemoglobin values ranging from 25 to 85 per cent (Sahli) were treated as follows.

1) Tetrachlorethylene, in a dosage of 0.12 ml./kg. (maximum dose, 5 ml.) was administered every 4 days until all worms were removed. On the evening before the treatment, patients had a light dinner without fats, and the drug was given next morning on an empty stomach.

2) Ferrous sulfate was given daily except on the day when tetrachlorethylene was administered, the dosage for adults being 3 × 0.25 gr. and for children, 2 × 0.25 gr. daily.

Patients were considered deparasitized when no hookworm eggs were found in the stools, by the method of Willis, on the 3rd and 4th days following treatment.

RESULTS

The efficacy of the above treatment was fully confirmed. The tetrachlorethylene was well tolerated, the only complaint being a slight epigastric burning sensation after swallowing the drug. As shown in Table 1, complete removal of worms was obtained in a little more than 95 per cent of the cases with 4 treatments or less, but as many as 7 treatments were needed to cure the last two patients. The intensity of infection was the principal factor influencing the number of treatments needed.

Hemoglobin regeneration began as soon as treatment was instituted, and was measured in 131 cases. Table 2 shows the amount of increase on the 40th day after the first treatment. In 7 patients with very low initial hemoglobin (25–45%), the increase was relatively small, lying between 10 and 30 per cent,

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TABLE 1

Number of courses of anthelmintic treatment necessary to remove worms, and its relation to the intensity of infection

No. of treatments	Eggs per gram of stool								Total cases	% cured by each treatment
	<1,000		1,000 to 5,000		5,000 to 20,000		>20,000			
	No. cured	%	No. cured	%	No. cured	%	No. cured	%		
1	41	46	13	28	2	9	1	17	57	35
2	28	31	18	39	3	13	4	66	53	32
3	16	18	10	22	1	4.3	0	—	27	16
4	4	4	4	9	12	52.1	0	—	20	12.5
5	0	—	1	2	3	13	1	17	5	3
6	0	—	—	—	1	4.3	—	—	1	0.5
7	1	1	—	—	1	4.3	—	—	2	1
Totals.....	90		46		23		6		165	

TABLE 2

Hemoglobin regeneration on the 40th day after the beginning of treatment, related to the degree of anemia

Initial Hgb. %	No. of cases	Per cent of hemoglobin increase													Av. gain.
		0	5	10	15	20	25	30	35	40	45	50	55	60	
25	1											1			
30	4						1		1				1	1	
35	2			1				1							36
40	3				1				2						
45	6			2	1						2	1			
50	10					5	2	2		1					
55	25		1	3	1	5	6	2	7						21
60	38			4	15	12	4	2	1						
65	21			6	2	5	8								
70	15		3	3	3	6									
75	4	1	1		2										16
80	0														
85	2		1		1										
Total.....	131	1	6	19	26	33	21	7	11	1	2	2	1	1	

but in 9 others it was more than 35 per cent and in one case reached 60 per cent. The average gain was 36 per cent. The 73 patients in the middle range of 50–60 per cent hemoglobin showed a slower improvement, averaging about 21 per cent, while the 42 less anemic cases, with hemoglobin of 65–85 per cent, made the least gain, averaging 16 per cent, including one case which made no gain at all.

As a check on the intensity of the infections, the number of hookworm eggs per gram of stool was estimated and correlated with the increase in hemoglobin

TABLE 3

Hemoglobin regeneration on the 40th day after beginning of treatment related to intensity of infection and degree of anemia

Initial hgb.	No. of cases	Eggs per gram of stool			
		<5000		>5000	
		Cases	Gain in hgb.	Cases	Gain in hgb.
25–35	7	5	36%	2	43%
36–55	43	31	23%	12	31%
56–75	81	71	16%	10	24%
Total cases and av. gains	131	107	19%	24	29%

after treatment (Table 3). As expected, the more worms, the greater the regeneration of hemoglobin. The patients with less than 5,000 eggs/gr. gained 19 per cent in hemoglobin in 40 days, while those with more than 5,000 eggs/gr. gained on the average 29 per cent. Table 3 also shows that where different intensities of infection caused the same degree of anemia, greater regeneration was noted in the cases with more parasites.

CONCLUSIONS

The omission of the saline purge after treatment of hookworm infection with tetrachlorethylene, as recommended by Carr *et al.*, increases the effectiveness of the drug, shortens the time of treatment, and lowers its toxicity and cost. This method thus facilitates the administration of mass treatment and improves the possibility of curing the disease.

REFERENCE

CARR, H. P., SARDÁ, M. E. P., AND NUÑES, N. A., 1954. Anthelmintic treatment of uncinariasis, *Am. J. Trop. Med. & Hyg.* **3**: 495–503.

THE RELATION OF PHYSIOGRAPHY TO THE TYPES OF FRESHWATER ENVIRONMENTS AND THE PRESENCE OF *AUSTRALORBIS GLABRATUS* IN PUERTO RICO[1]

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In Puerto Rico, *Australorbis glabratus* is considered the sole vector of *Schistosoma mansoni*. The irregular distribution of this snail and of schistosomiasis presents problems of an ecological nature which require a comparative approach for their solution. In April, 1953, surveys were started directed toward determining the types of freshwater environments present, their physical, chemical and biotic characteristics, and to consider what effect these might have on the transmission of schistosomiasis.

While much has been published on the distribution of schistosomiasis in Puerto Rico, the climate, and topography of the island, there has been little correlation of these factors and their possible relationship to the epidemiology of the disease. Hoffman (1927), Hoffman and Faust (1934) and Faust *et al.* (1934) discussed the general incidence in man, and initiated studies on the biology of the snail and ecology of freshwater habitats. The last two, unfortunately, have received little attention since this early work. Pons (1937), Oliver-González *et al.* (1950), Hoffman and Janer (1939) and Serra (1930) reported studies made on the incidence in man in limited areas. Weller and Dammin (1945) provided the most recent comprehensive account. They examined 19,139 men aged 18 to 38 years and determined the incidence of schistosomiasis. Although this group was limited by sex and age it included persons from each of the 76 municipalities of the island. These political districts show only a general correlation with physiography, and consequently the kinds of freshwater environments of a given area. As frequently occurs when reporting the incidence of schistosomiasis, in Puerto Rico and elsewhere, large areas may be incriminated where only a small part of the political unit may actually be involved. The incidence of schistosomiasis in the various areas referred to in the present paper is that given by Weller and Dammin, unless otherwise noted.

The physical characteristics of freshwater environments, to which we shall limit ourselves in the present account, are primarily the result of climate and topography. The climate has been adequately studied by Fassig (1928, 1929). Meyerhoff (1933) has given the most recent comprehensive account of the geology of the island; Roberts (1942) has discussed the interrelationship of climate and geology to the soils of Puerto Rico; and Pico (1950) has reviewed all of these factors in relation to the human geography of the island. For present

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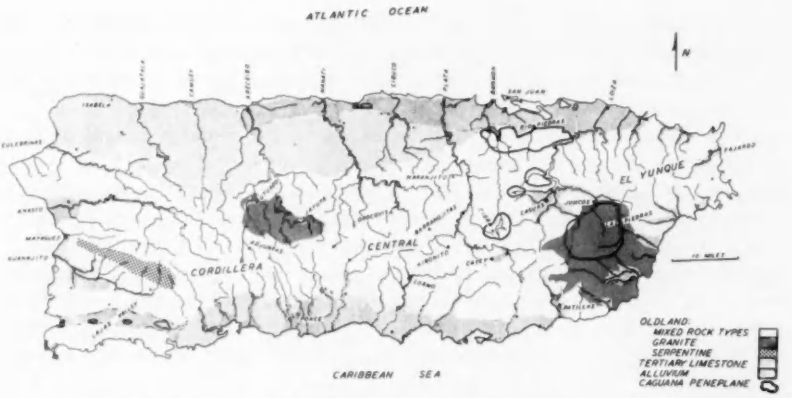

FIG. 1. Geological map of Puerto Rico. Many of the smaller areas of plutonic rocks, upland alluviated valleys and Caguana Peneplane have been omitted.

purposes it is convenient to recognize three major physiographic regions in Puerto Rico (Figure 1): (1) the Cretaceous Oldland Province, consisting chiefly of well indurated volcanic and plutonic igneous rocks, but including limited areas of shale and limestone; (2) the Tertiary Limestone Province; (3) the Quarternary Alluvial Province, which is very discontinuous and largely dissects the other two provinces.

THE CLIMATE

The prevailing winds in Puerto Rico are from the northeast. Within limits, areas of low elevation receive rainfall in direct proportion to the extent of land in line with the prevailing winds. Rainfall increases with altitude, and areas immediately to the windward of higher elevations receive it in proportion to their proximity to the elevation, and the height of the latter. Areas to the leeward of the higher elevations tend to be arid.

The mountainous El Yunque area, which has an altitude of 3,500 feet, receives an average rainfall of 200 inches at its summit, while the northeastern tip of the island, only 12 miles to the windward and at 150 feet elevation, gets less than 50 inches. The Caguas Valley, in spite of being immediately leeward of El Yunque, receives between 60 and 70 inches because it is windward to part of another high mountain mass, the Cordillera Central. This great monadnock crest extends from the Caguas Valley almost to the west end of the island, and parallels the south coast within 10 miles of it. Elevations of 3,000 to 3,500 feet are common along this range, and with El Yunque represent the maximum in Puerto Rico.

Of the three physiographic provinces on the island, the Oldland Province receives the most rainfall because of its relationship with the prevailing winds and higher altitude. Almost all of it receives more than 70 inches per year. The Tertiary Limestone belt and alluvial areas of the north coast receive between 60 and 70 inches, with the exception of the portion west of the Manati River where

the rainfall is somewhat less. The Tertiary Limestone area of the south coast receives between 30 and 40 inches, and all of the alluvial areas west of Guayama on the south coast receives 30 to 50 inches, decreasing from east to west and from north to south.

The seasonal variation of rainfall in Puerto Rico is usually spoken of as an alternation of wet and dry seasons. There is a dry period extending from December through April; rainfall increases in May; there is a second dry season in midsummer; and the heaviest rainfall occurs from August through November during the hurricane season. Usually rain occurs as showers of varying intensity and only a few hours' duration. In the arid section along the south coast rainfall may be only one inch per month during the winter dry season and 4 to 8 inches during the wet season. In the more humid areas north of the Cordillera Central a minimum of 4 and a maximum of 16 inches per month typifies the seasonal variation.

Monthly average temperatures show that at any one locality there is scarcely 3°–5° C. variation throughout the year. The period from December to April is invariably cooler than the rest of the year regardless of locality. The average monthly temperatures on the island range from 19° to 23° C. on El Yunque to 25° to 28° C. at Guayama. Exceptions beyond these monthly averages are few and of short duration. Diurnal variation is usually a few degrees greater than seasonal averages.

The pattern of rainfall and temperatures has a marked effect on the freshwater environments. As opposed to what is seen in temperate continental areas, in Puerto Rico the streams do not develop pronounced volume and temperature curves that extend through a greater part of a season. There is a brief flushing of the streams immediately following a shower, varying with the intensity of the rainfall, but the stream returns to a stage which may be called the "normal low" within a few hours. The physical effect of flushing, which tends to wash out the snails, is accompanied by changes in water quality, chiefly in the reduction of the amount of dissolved solids. There is some indication that the latter has an adverse effect on the snails (unpublished data).

The alternation of wet and dry phases of an aquatic environment, correlated with the seasons, is a phenomenon limited chiefly to the irrigation tanks of the south coast. In these, the oscillation of the water level is controlled by man. Such situations do not seem to contain *Australorbis* but may have a related species, *Tropicorbis riisei*[3]. Natural temporary environments, which are regularly in an aquatic phase for a period less than a year, also do not maintain *Australorbis* populations in Puerto Rico. Small temporary puddles, a few inches deep and only a few feet in maximum surface dimension, are found in all parts of the island. These appear in deep ruts of dirt roads, near road embankments, and similar spots where the natural contour of the land has been modified. They serve as the habitat for *Tropicorbis decipiens*[3], a small planorbid snail sometimes confused by

[3] Pending completion of our studies on the systematics of the freshwater mollusca of Puerto Rico, the nomenclature of the most recent comprehensive list, van der Schalie (1948), will be used

the uninitiated with juvenile *Australorbis glabratus*. This snail seems never to occur as perpetuating colonies in other types of environments.

THE OLDLAND PROVINCE

The substrate of the Oldland Province is made up chiefly of small units of diverse volcanic rocks, plus some small areas of granite, serpentine, shale and limestone. The heterogeneity of rock type, often changing within a few hundred meters, is such that a single type of rock rarely imposes its own peculiar characters on a drainage system. However, two areas of granite and one of serpentine (Figure 1) are sufficiently extensive and of such a nature that distinctive stream types develop on them.

Topographically, most of this Province is dominated by the St. John Peneplane, which slopes gently upward from 1,600 feet elevation in the east to 3,000 feet in the west. The monadnocks along its border, El Yunque and the Cordillera Central, extend several hundred feet higher. The original surface of this peneplane is now represented only by the coordinated heights of sharp ridges forming the drainage divides. A second erosional surface, the Caguana Peneplane, is found at an elevation of one hundred to several hundred feet. Its variation in elevation somewhat parallels that of the St. John Peneplane. There

FIG. 2. Looking eastward across the Caguana Peneplane at Las Piedras (background). The small streams (indicated by clumps of bamboo) contain *Australorbis*. The St. John Peneplane and its monadnocks constitute the skyline.

are several areas where the original surface of this peneplane are more or less intact. Such areas form broad, flat or gently rolling surfaces, surrounded by elevations reaching up to the St. John Peneplane (Figure 2).

Types of freshwater environments. Throughout the Oldland Province the general slope is sufficiently steep and the geological history is such that swift-running water providing lotic environments are characteristic. In a given area the dominating peneplane surface furnishes a clue to the steepness of the gradient of the smaller streams. The predominating rock type (mixed, granite or serpentine) determines the stream's substrate material and the quality of the water. Streams flowing down the valley walls from the St. John Peneplane, or the monadnocks above it, generally have steep gradients but are relatively permanent because of the high rainfall of the region. Short slackwater reaches are separated by steep riffles or falls, and the substrate is chiefly of bedrock and large angular boulders. The biota of these streams is limited in species and individuals, and *Australorbis* is generally absent.

In the larger areas dominated by the Caguana Peneplane, the streams are generally entrenched in V-shaped valleys 20 to 200 feet below the original surface. The streams have a lower gradient than those flowing down from the St. John Peneplane. Biotically they are richer in species and individuals, and *Australorbis*

FIG. 3. A typical granite stream, the Rio Valenciano at Juncos, downstream from Figure 2. Photo by Atilles, courtesy of the University of Puerto Rico Agricultural Extension Service.

is often present. Long slackwater reaches are separated by short, gentle riffles, and the substrate material is chiefly of smaller sediments.

Around Las Piedras, south of the Caguas Valley, a broad development of the Caguana Peneplane is underlain almost entirely by granite (Figures 1 and 2). Streams of this area which average less than 20 feet in width are typical of those in other parts of the Caguana Peneplane, and contain *Australorbis*. As the streams become larger, however, the distinctive features of streams from granite areas become more apparent. The formation of boulders from the granite bedrock is relatively uncommon, in contrast to what is found in areas of well indurated volcanic rocks. Instead, weathering is chiefly by the separation of the component crystals, a millimeter or two in maximum dimension, and thus the bedrock is converted almost directly into coarse sand. The channels of larger granite streams are filled with this sand (Figure 3). Channel-braiding is frequent, but almost no riffle areas are found. The gentle flow of the clear, shallow water is sufficient to keep the substrate continually in motion during most of the year. Sedentary organisms such as submerged plants and snails are generally not present as established populations. However, during very dry periods, the water level may fall even below the usual "normal low" stage, and there is less shifting of the substrate material. On three such occasions infected *Australorbis* have been found at the stream shown in Figure 3, and it is believed they may have floated down from the headwater area (Figure 2), where they are abundant. In the part of the stream shown, they find temporary shelter in rubbish (cans, boxes, tires, etc.).

Granite represents a type of rock which easily undergoes erosion in the tropics. Associated with the two major granite areas of Puerto Rico (Figure 1) are some of the larger upland alluviated valleys and more extensive developments of the Caguana Peneplane, both of which provide very favorable snail habitats. The single major serpentine area seems to have been resistant to erosion, and much of it remains as a high ridge. The streams flowing entirely on this serpentine have a high gradient. Except for this characteristic, they remind one of the streams of the Limestone Province (v.i.), for their substrate consists of bedrock with only a few boulders, and springs are frequent. There is a dearth of aquatic organisms. *Australorbis* has not been found in these streams.

A number of artificial lenitic environments of quiet water have been produced in the Oldland Province by damming streams. These reservoirs range in size from mere diversionary dams to large lakes several miles long. *Helisoma* sp., *Tropicorbis riisei*, *T. albicans*, *Physa* sp., *Lymnaea* sp. and *Uncancylus* sp. have been found, usually in limited numbers, in the major reservoirs examined (Loiza, Caonillas, Dos Bocas, Cidra, Coamo, Guineo, Garzas, Curias, Guayabal, Patillas). No evidence has been obtained to indicate that they contain established populations of *A. glabratus*. The reason for the exclusion of this snail is not clear but water quality may be a significant factor. The water of the reservoirs in the Oldland area tends to be more dilute than most waters in which the snail is known to occur in Puerto Rico. Seasonal fluctuation of the water level is another possible limiting factor. In some reservoirs, as Guayabal and Patillas, the bank

FIG. 4. Dos Bocas reservoir, on the Rio Arecibo downstream from Utuado. Photo by Atilles, courtesy of the University of Puerto Rico Agricultural Extension Service.

slopes 20° or more, and the shore line fluctuates in tens of feet annually. In reservoirs like Dos Bocas (Figure 4) and Curias, however, the water level remains relatively constant and a prominent zone of aquatic vegetation has developed along their margins.

The reservoirs can be divided into two groups according to the presence or absence of *Australorbis* and schistosomiasis in their headwater areas. The headwaters of the Curias, Guineo, Garzas and Yauco reservoirs are all high gradient streams, potentially poor *Australorbis* habitats. The Loiza, Cidra, La Plata, Dos Bocas and Caonillas reservoirs have headwater areas where *Australorbis* can generally be demonstrated and in which the incidence of schistosomiasis in man is moderate to high. The latter headwater areas are alluviated upland valleys and/or extensive developments of the Caguana Peneplane surface. While there seems to be little likelihood that *Australorbis* will become established in any of these reservoirs, it is possible that under certain conditions cercariae and infected snails may float downstream and enter them.

Schistosomiasis in the Oldland Province. The areas of broad development of the Caguana Peneplane are the chief foci of schistosomiasis within this physiographic province. For example, the municipalities of Aguas Buenas and Las Piedras are primarily of this physiographic type and the reported incidence in man is 30 and 41 per cent, respectively. Unfortunately it is impossible to be

certain in those cases whether these infections were acquired on the peneplane or in the alluvial areas which adjoin them. The municipality of Cidra contains a large Caguana Peneplane surface without an alluvial valley adjoining it, and 15 per cent of the men examined were found infected. By contrast, the incidence tends to be low or absent in municipalities predominantly on the St. John Peneplane: e.g., Maricao, 2 per cent; Orocovis, 1 per cent; Naranjito, 1 per cent; and Las Marias, negative.

THE TERTIARY LIMESTONE PROVINCE

South coastal area. Along the north and south coasts in the western part of the island there are horizontal beds of Tertiary limestone several hundreds of feet thick. The maximum elevation of the southern limestone area is about 200 meters and it has differences of elevation of 100 meters in 1,000 meters horizontal distance. Karst topography is not prominent and none of the occasional sinks contains ponds. The area is divided into a number of units that are separated by alluvial valleys along the streams flowing from the southern flank of the Cordillera Central (Figure 1). This is one of the dryest regions of Puerto Rico, with 30–40 inches of rain per year, and a temperature which remains within a few degrees of 25°C. No permanent streams or springs were found in the area and cactus is prominent in the bush-covered, sparsely populated hills. The absence of freshwater habitats precludes *Australorbis* and the transmission of schistosomiasis.

North coastal area. The maximum width of this area is about 10 miles (Figure 1). Most of it is over 100 meters in elevation and in places it rises to 400 meters. Karst topography is much exaggerated, with steep-sided hills resembling haystacks surrounding isolated depressions. The temperature is similar to that found on the south coast but rainfall is higher, ranging from 50 to 70 inches per year.

In contrast to the Oldland Province, the process of erosion in the Limestone Province is chiefly chemical rather than mechanical. Drainage is mostly underground. The few surface streams have almost no rubble in them and bedrock predominates as the substrate type. Their gradient is low. These streams tend to have impoverished biotas, and *Australorbis* has not been found in them.

Natural lenitic environments, in the form of limestone-sink ponds, are numerous and vary in size from a few square feet to several acres in extent. The water is usually dark colored, occasionally turbid, and the shores are often marshy. *A. glabratus* is present in perhaps half of these ponds but most populations are not dense. Relatively heavy populations sometimes occur in ponds where cattle are watered. The irregular distribution of snails in these ponds is apparently correlated with the great diversity of chemical and biotic conditions present. Other than the occasional use for watering stock, these ponds are little used by man.

A large artificial reservoir on the Guajataca River rests entirely on Tertiary limestone. Unlike the reservoirs on the streams in the Oldland Province, the quality of the water here has a total dissolved solids concentration well within the range we have found in the *Australorbis* habitats on the island. There is a fluctuation of water level of several feet during the year but the shallower depths

support an abundance of *Najas guadalupensis*, a plant that is elsewhere frequently associated with the snail. An open aqueduct takes water from the reservoir to the northwestern part of the island, in the vicinity of Isabela. It was surprising to find *A. glabratus* and other species of planorbid snails in this aqueduct, since they have not been found in the reservoir. The aqueduct is used for washing clothes, bathing, and as a source for household water. In its lower reaches it supplies several tanks (farm ponds). The biota and quality of water in these ponds are similar to the tanks of the south central coastal alluvium (v.i.). In one of them, however, *A. glabratus* was found in limited numbers.

Schistosomiasis potential in the limestone area. The limestone sink ponds in Puerto Rico do not seem to be an important source of schistosomiasis, even though *Australorbis* may be present. Examination of snails from more than a dozen ponds failed to show any infections. This confirms the earlier work of Hoffman and Faust (1934). Surveys of human populations in the area have usually shown an incidence of less than 3 per cent. The infrequency of human contact with the ponds may be responsible for the low incidence of the disease.

In 1945 Weller and Dammin reported 6 per cent incidence in 284 men from the municipality of Isabela, thus attributing to it the highest in the Limestone Province. Only six years earlier Hoffman and Janer (1939) found less than 1 per cent incidence in 7,052 persons of that municipality. The terminal branches of the Guajataca aqueduct, built in 1921, are distributed around Isabela, and this might be cited as a potential cause of a higher incidence here than elsewhere in the Limestone Province. However, we are inclined to think some discrepancy in sampling techniques accounts for the differences between the two reports.

THE ALLUVIAL PROVINCE

Most of the alluvium in Puerto Rico is found at the mouths of rivers along the coast, but there are also upland alluviated valleys that are nearly or completely disjunct from the coastal alluvium. The coastal alluvium of adjacent streams may be isolated or continuous, and narrow extensions of it may bound a stream for several miles into the other provinces (Figure 1).

Because of the slight regional uplift since the alluvial areas were formed, the streams are now entrenched on the sediments they had formerly deposited. Alluvial terraces are conspicuous. As a result there is no regular annual flood stage that inundates the surrounding land, as found in some endemic areas for schistosomiasis, e.g., Egypt and China. Brief, irregular periods of extreme flooding may occur during the hurricane season, but these last only a few days at most and occur less than once a year. Moreover, the entrenchment has resulted in steeper gradients than one might expect on streams margined by alluvium. Many reaches of such streams are steeper than any which contain *Australorbis*. The larger streams tend to meander, but this phenomenon is not as frequent as it would be if the streams were aggrading. The alternating series of backwater areas associated with sediment bars opposite undercut banks are rare and poorly defined. Disjunct oxbows are also rare but disjunct braids are relatively common. *Australorbis* is often found in such situations.

Natural levees are not prominent but there is a marked tendency for tributary streams to flow downstream along the alluvium before entering the main stream. This produces long reaches of low gradient and *Australorbis* is often found in the tributaries when they are absent or rare in the main stream. Good examples of these "yazoo" tributaries are present at Arecibo (Figure 6), Fajardo and Patillas. At the lower ends of major streams, in the angle formed by the ocean beach and the natural levee, estuarine marshes frequently develop. These generally have a small stream (caño) draining them. The caños and marshes are often spring fed where the alluvium is associated with limestone, shale or serpentine. *A. glabratus* rarely occurs in the springs at their source but is frequently found in the associated marsh and stream. Usually the lower parts of the estuarine marshes are subject to tidal fluctuation. The schistosomiasis vector is generally absent in the tidal marshes, but sometimes present beyond the tidal limit. Biotic zonation, chiefly shown by the aquatic plants, is a useful clue to the sections likely to contain the snail.

Sugar cane is extensively grown on the alluvial areas. This plant has definite soil moisture requirements and several artificial freshwater habitats have been produced to meet them. In the estuarine marshes drainage ditches have been dug and caños have been straightened and dredged. These often become choked with vegetation and frequently serve as *Australorbis* habitats. In other areas, where the rainfall averages less than 60 inches a year, it is necessary to irrigate. Attempts have been made to irrigate in three areas along the north coast. These have been abandoned and at present all irrigation of cane is confined to the southern coastal alluvium. Details on the latter are given below.

There are several natural freshwater lakes on the coastal alluvium. Lake Tortuguero, between the Cibuco and Manati Rivers on the north coast, is one of a series of remnants of an ancient marine lagoon. It has a distinctive biota (*Najas marina*, *Palaemon pandaliformis*). The water has a high dissolved solids content and a pH of 9.1, a water quality scarcely duplicated elsewhere on the island. The fine grained, organic silt bottom is heavily impregnated with precipitated calcium salts. The lake receives drainage ditches from the surrounding marsh, which contain *A. glabratus* and several other species of freshwater snails. No mollusks have been found in the lake.

Streams on alluvium tend to show distinctive features according to the major rock type in their headwater area. Those originating in the mixed rock types of the Oldland develop ragged margins, with substrates of rounded conglomerate boulders, gravels, and a large array of finer sediments (Figure 5). Even the major streams (Plata, Loiza, Añasco) tend to be shallow, averaging less than two feet in depth. The last mile of their reaches becomes deeper, subject to tidal fluctuation, and brackish.

Streams having headwater areas chiefly of granite remain shallow and braided, with coarse sand bottoms. Some of the lateral braids have become disjunct and biotic succession has gradually produced suitable habitats for *Australorbis*. The Arecibo River near its mouth furnishes examples of this phenomenon on a large scale (Figure 6). Other streams of the granite type are those on the alluvium

FIG. 5. The Rio Gaudiana at Naranjito. A typical stream from mixed type rocks of the Oldland, at its upper extent on alluvium. Note the ragged margin, rounded conglomerate boulders and encroaching marginal vegetation. However, no *Australorbis* occurs at this station.

at Humacao, Yabucoa, and Manuabo, and some streams in the Caguas and Utuado Valleys. While the main channels of the larger granite streams of these areas do not harbor permanent populations of the snail, seepage areas, disjunct pools and estuarine marshes do so in all the places mentioned.

Limestone streams on alluvium are represented by the Guajataca, Camuy and Cibuco Rivers. In these streams the channels are deep in proportion to their width, and pools, braiding and ragged margins are absent. *Australorbis* is not found in the main channel of these streams, but is present in the estuarine marshes at their mouths.

From the Bayamon Valley to that of the Loiza River, in the vicinity of San Juan Bay, there is a coastal area of alluvium with distinctive ecological features. It is margined inland by thin bedded, well indurated Cretaceous shales which are broadly beveled by the Caguana Peneplane. Most headwater streams are of low gradient and abundant springs insure a permanent flow. Water cress, the only aquatic plant cultivated on the island, is grown along some of the streams in this area. *A. glabratus* is frequently present in the cress beds. Marine lagoons along the coast are margined inland by extensive brackish and freshwater marshes. Several small streams flow independently into the lagoons, without becoming consolidated into the major drainage systems of the area (Piedras, Loiza and

FIG. 6. Looking westward from the mouth of the Rio Arecibo: A. Tertiary limestone of the North Coast. B. A small yazoo tributary (with infected *Australorbis*) on the alluvium of the main stream. C. Abandoned, plant-filled channel of the main stream, favorable breeding sites for *A. glabratus*. D. Functional channels of the main stream, in which snails may occur accidentally. An extensive estuarine marsh (Tiburones) is immediately east of the scene. It contains many situations favorable for the snail. Photo by Atilles, courtesy of the University of Puerto Rico Agricultural Extension Service.

Bayamon Rivers). *Australorbis* has a spotty distribution in the streams and freshwater marshes of the area, and rarely occurs in dense populations. The human population here includes San Juan and suburbs and is the most dense in Puerto Rico. This has long been considered an important focus for schistosomiasis. Weller and Dammin (1945) reported that the incidence ranged from 3 to 16 per cent. Oliver-González *et al.* (1950) found 44 per cent in a small community along the Sabana Llana Creek.

Because of the lower rainfall, freshwater habitats on the alluvium of the south coast differ markedly from those discussed above. The Lajas Valley, in the southwestern part of the island, is unusual in that it does not have a major stream. The small tributary streams flowing down the northern valley wall have produced alluvial fans sloping southward and three broad, shallow lakes (Cartagena, Anegado, Guanica) have developed along the southern margin of the valley. The first two are more permanent than Lake Guanica and are choked with freshwater vegetation. Both contain *A. glabratus*. When Lake Guanica was first visited in 1953 it had recently started a new aquatic phase and no snails were

present. *Australorbis* was found, however, in the spring fed seepage areas around the shore. A heavy population of *Tropicorbis riisei* was found when the lake was examined in 1954, but *Australorbis* was still absent. Drainage and irrigation ditches in this valley harbor the latter snail.

Between Patillas and Ponce some of the major streams flowing down the south flank of the Cordillera Central have permanent reaches on fingers of alluvium extending into the Oldland Province (Jacaguas, Lapa, Coamo and Patillas Rivers). All of them become dry arroyos when they reach the broad coastal alluvium. Water flows in the arroyos only for a few weeks during the height of the rainy season. The major reservoirs on these rivers have been discussed in connection with the Oldland Province. Diversionary dams or deep wells are used to fill tanks, the common freshwater habitat on this coastal alluvium. These vary in size from a few hundred feet in their maximum dimension to many acres in extent. Those depending on rainfall show marked changes in water levels. Such pounds tend to be biotically barren. Tanks that maintain a rather constant water level, usually those filled from wells, develop an abundant biota.

The distribution of *Australorbis* in the Southern Coastal Alluvium is peculiar. The snail is found in some low gradient streams on fingers of coastal alluvium, e.g., the Patillas River and some smaller streams in the vicinity of Guayama and Patillas. It is also found in the major aqueducts of the region. But it is apparently not present as perpetuating populations in most tanks. A small planorbid snail, probably *Tropicorbis riisei*, occurs in great abundance in many of the tanks and some of the low gradient streams (Guamani, Lapa, Coamo). This snail may have led to the indications by Hoffman and Faust (1934) that they were somewhat doubtful about the identity of their material from this area. In a few places this species and *A. glabratus* occur together. While *T. riisei* may be synonymous with *T. havanensis*, a species that has been experimentally infected with *S. mansoni* (Brooks, 1953), our own attempts to infect Puerto Rican *T. riisei* have been unsuccessful.

Schistosomiasis in the alluvial province. Previous surveys of the incidence of schistosomiasis in man have shown that some of the alluviated areas are among those having the highest on the island. Other parts of the alluvium, however, have such a low incidence that it is thought the infections were acquired elsewhere. An extended account of the various subdivisions of the Alluvial Province and their relationship to the epidemiology of the disease is beyond the scope of the present paper. They vary according to the climiate, types of aquatic situations present, and the character of the rocks from which they have developed. The basic information has been given in the above section. *A. glabratus* is common in most upland alluviated valleys and these are also centers of human population (Caguas, Cayey, Barranquitas, Aibonito, Jayuya, Utuado). All of these have a moderate to high incidence of the infection. In the present study the heaviest populations of *A. glabratus* on the island were found in small streams at Cayey and Caguas. The combination of ecological factors, presence of snail colonies, and their proximity to human populations have conspired to make these some of the most important centers of schistosomiasis in Puerto Rico. The only other areas

that approach them in significance are a few areas on the coastal alluvium and the Caguana Peneplane.

SUMMARY

The differentiation of the kinds of habitats favorable or unfavorable for *Australorbis glabratus* is an initial step in seeking the essential factors associated with its distribution. It is also possible that such basic considerations may be of value in the control of schistosomiasis. In Puerto Rico the snail occurs in some low gradient streams, major aqueducts, disjunct marginal pools along streams, the estuarine marshes and limestone sink-ponds. It is absent, as perpetuating populations, in the major reservoirs, most artificial irrigation tanks, temporary flowing and nonflowing waters, in environments which have tidal fluctuation, in high gradient streams on any rock type, and in low gradient streams on limestone. The correlation of the various types of freshwater environments with the climate and physiography have been discussed. Schistosomiasis is not regularly transmitted in some areas where the snail occurs, e.g., the limestone area of the north coast. It is not known whether this is due to infrequency of human contact or some other factor. The occurrence of the snail vector in some isolated freshwater environments emphasizes that it is capable of crossing barriers to invade new habitats by passive dispersal.

ACKNOWLEDGMENTS

We are grateful to Dr. Donald B. McMullen for his encouragement during the study, and critical review of the manuscript. To Dr. José Maldonado of the University of Puerto Rico School of Medicine, and Dr. David Pimentel of the U. S. Public Health Service we are indebted for access to their recent data, as yet unpublished, on the present incidence of schistosomiasis in selected areas of Puerto Rico. Figures 3, 4 and 6 were provided by the University of Puerto Rico Agricultural Extension Service.

REFERENCES

Brooks, C. P., 1953. A comparative study of *Schistosoma mansoni* in *Tropicorbis havanensis* and *Australorbis glabratus*, *J. Parasit.* **39**: 159–163.

Fassig, O. L., 1928. The climate of Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **4**: 199–211.

Fassig, O. L., 1929. On the average monthly and annual rainfall of Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **5**: 332–343.

Faust, E. C., Hoffman, W. A., Jones, C. A., and Janer, A. L., 1934. Studies on schistosomiasis in Puerto Rico. II. The epidemiology and geographic distribution of schistosomiasis mansoni in Puerto Rico. 2. A survey of intestinal parasites in endemic schistosomiasis areas in Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **9**: 447–471.

Hoffman, W. A., 1927. Studies on schistosomiasis (*S. mansoni*) in Puerto Rico. 1. Preliminary report on the distribution of *S. mansoni*, *Puerto Rico Review of Pub. Health and Trop Med.* **3**: 223–230.

Hoffman, W. A. and Faust, E. C., 1934. Studies on schistosomiasis in Puerto Rico. II. The epidemiology and geographical distribution of schistosomiasis mansoni in Puerto Rico. 1. Epidemiology of the infection on the island, *Puerto Rico Jour. Pub. Health and Trop. Med.* **9**: 228–254.

HOFFMAN, W. A., AND JANER, J. L., 1939. A parasite survey of Isabela, *Puerto Rico Jour. Pub. Health and Trop. Med.* **14**: 439–443.

MEYERHOFF, H. A., 1933. Geology of Puerto Rico, *Monographs of the University of Puerto Rico*, Ser. B, No. 1, 306 pages.

OLIVER-GONZÁLEZ, J., BIAGGI, N., ACEVEDO, C., MARIOTTA, D. A., HERNANDEZ-MORALES, F., SANTIAGO-STEVENSON, D., AND PEREZ-SANTIAGO, E., 1950. Studies on Manson's schistosomiasis in Puerto Rico. I. Results of control methods in an endemic area, *Puerto Rico Jour. Pub. Health and Trop. Med.* **25**: 387–398.

PICO, R., 1950. *The geographic regions in Puerto Rico*, Univ. Puerto Rico Press, San Juan, 256 pages.

PONS, J. A., 1937. Studies on schistosomiasis mansoni in Puerto Rico. V. Clinical aspects of schistosomiasis mansoni in Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **13**: 171–254.

ROBERTS, R. C., 1942. *Soil survey of Puerto Rico*, U. S. Dept. Agriculture Ser. 1936, No. 8, 503 pages.

SERRA, A., 1930. Helminths in southern Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **6**: 91–105.

VAN DER SCHALIE, H., 1948. *The land and freshwater mollusks of Puerto Rico*, Museum of Zoology, Univ. of Michigan Misc. Pub. No. 70: 1–134.

WELLER, T. H., AND DAMMIN, G. J., 1945. The incidence and distribution of *Schistosoma mansoni* and other helminths in Puerto Rico, *Puerto Rico Journal of Pub. Health and Trop. Med.* **21**: 125–147.

CORRESPONDENCE

March 19, 1956

To the Editor:

The interesting paper by Magath and Thompson (Amer. J. Trop. Med. Hyg. **4**: 941–946, 1955) illustrates the assumption that well conceived and brilliantly executed research may not always suggest a philosophy consistent with the public health implications of that research. The peculiar public health philosophy which these authors propose is the sole reason for the following remarks, which are my personal opinions.

The epidemiology of trichinosis suggests that subclinical infection in humans produces an effective immunity which prevents severe clinical episodes and even death. How long this immunity is effective is not known. On this basis, it is suggested that the irradiation of the parasite in pork to prevent infection in humans might ultimately result in high mortality rates in man since the establishment of immunity would be precluded. Thus the authors conclude that "until a specific cure for the disease is discovered", irradiation of pork "... might prove hazardous."

This view reminds me of the opinions of doctors in West Africa, whom I met during World War II, who argued that malaria was a disease with which one should learn to live, and that preventive malaria control was a fanatical practice somewhat in the temper of the American love for shower baths and cleanliness. But can we say, for instance, that higher standards of sanitation and personal hygiene should be largely abandoned because they destroy the possibility for the acquiring of immunity to certain viral infections? This notion, it seems to me, insists that *any* measure, short of a specific cure, which will prevent infection by the trichina worm is an impractical measure since "a little infection" is good for the population.

I feel this philosophy to be inconsistent with the objectives of public health. The major aim of public health is the prevention of infection and disease acquired naturally. This entertains the dual objective of ultimate protection from and elimination of the etiologic agents of disease or the vectoral pathways by which they are conveyed to man. On this basis, I suggest that scientific workers in public health cannot admit the necessity of naturally acquired subclinical infection as a prophylactic measure. If infections are permitted to exist at any level, the rationale for sound public health practice becomes very tenuous.

A dynamic public health approach to this problem is one in which research simultaneously seeks solutions on all logical fronts of attack. Thus an attitude should be favored which looks for ultimate control on the basis of a wide program of eliminating the sources of infection by any safe means, education of the public, and at the same time developing better vaccines and anthelmintics that might prove of practical value. If subclinical infection is immunologically beneficial to humans, one avenue of research might be to provide an infection derived under strict scientific laboratory control. This would negate the rather

obsolete principle of dependence upon infections subject to the unpredictable fluctuations of a disease agent in its natural state.

Sincerely yours,

LEO KARTMAN
Communicable Disease Center
U. S. Public Health Service
San Francisco, California

April 6, 1956

To the Editor:

We certainly cannot disagree with Dr. Kartman that the ultimate goal of public health practice is the prevention of infection and disease acquired naturally—and we might add, artificially also. We are sorry if Dr. Kartman read into our conclusion any difference of opinion on this point. We thought the expression "might prove hazardous" suggested our own lack of acceptance of the thesis that no one should look for a better method of control or that our suggestions were proved facts.

Until now, no serious advances in the control of biologic infections of trichinae have been made since that early public health officer, Moses, began his campaign. All evidence points to the fact that in the United States the rate of infection certainly is not decreasing. The annual rate of clinical trichinosis has remained about level, after falling from higher levels of 75 years ago. Indeed, the number of clinical cases can scarcely command enough attention to justify large expenditures of money for prevention, especially in view of the fact that the simple expedient of thorough cooking of pork will act as a completely preventative measure.

We also should like to see a "dynamic public health approach to the problem." So far the only dynamic (*sic!*) approach to this problem has been a regulation, dating years back, by the Bureau of Animal Industry, which requires that only pork or pork products not "customarily well cooked" must be processed to kill trichinae in those slaughterhouses in which federal inspection of meat is practiced. The most recent regulations forbidding the interstate shipment of hogs which have been fed raw garbage was not an antitrichinosis measure. Neither was it a regulation promulgated by the U. S. Public Health Service.

We suspect the most potent protection of the public has always resided in subclinical, naturally acquired infections, and that these infections have produced an immunity to many diseases. These subclinical, naturally acquired infections certainly are the reason many adults escape infectious childhood diseases. We dare say that Dr. Kartman would not suggest that up until 1954 anything other than naturally acquired immunity protected the vast majority of individuals from poliomyelitis. In spite of the greatest public health campaign ever experienced in the history of mankind, only a small percentage of the population has been yet artificially immunized against this disease, and the vaccine remains in short supply. It is certain that if we had to depend on diphtheria immunization alone, many of us would be dead of the disease. Dr.

Kartman's parallel of malaria hardly applies, for all evidence indicates (and we refer to animal experiments and postmortem observations) that a very few trichinae will confer permanent immunity without causing any clinical symptoms, whereas a severe and near-fatal infection with plasmodia does not confer any lasting, demonstrable immunity.

We most heartily agree that every avenue of scientific adventure should be traveled in the search for a good method of prevention and treatment of trichinosis, but we happen to doubt the practicability of irradiation of all pork and pork products. Even under the most favorable conditions, the present cost per pound of such a procedure exactly equals the profits of the meat industry on the product, and furthermore, considerably less than half of the pork consumed, even in the United States, is slaughtered in federally inspected plants. All attempts at education of the public, and there have been many, thus far appear to have produced little effect on the incidence of biologic trichinosis—man is basically a meat eater and he likes it rare!

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and
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BOOKS RECEIVED

Medical Parasitology for Medical Students and Practicing Physicians, by William G. Sawitz. 342 pp. illustrated. New York: McGraw-Hill Book Company, Inc., 1956. $6.00.

Dictionary of Dietetics, by Rhoda Ellis. 152 pp. New York: Philosophical Library, 1956. $6.00.

Veterinary Parasitology, by Geoffrey LaPage. 964 pp. illustrated. Springfield, Illinois: Charles C. Thomas, 1956. $12.50.

An Atlas of Regional Dermatology, by G. H. Percival and T. C. Dodds. 264 pp., illustrated with 475 in color. Baltimore: The Williams and Wilkins Co., 1955. $19.00.

Annual Review of Entomology, Volume I, 1956, ed. by E. A. Steinhaus. 466 pp. illustrated. Stanford, California: Annual Reviews, Inc., in cooperation with Entomological Society of America, 1956. $7.00.

BOOK REVIEWS

A Review of Work on Indian Medicinal Plants—Special Report Series No. 30, Indian Council of Medical Research, by R. H. Chopra and I. C. Chopra, 321 pp., New Delhi, 1955; Price Rs 3/-.

This review reflects the efforts of the Indian Council of Medical Research to support studies of indigenous drugs over India and elsewhere. In 1939 the senior author published the results of Indian efforts supported by the Council. The present "telegraphic" exposé lists plants used in indigenous systems of therapy, as well as references to both Indian and Western sources. The ten chapters include poisonous plants as well as those "used as remedies." Summaries of work done on commonly used drugs include one on "*Rauwolfia serpentina*" and its introduction into Western medicine.

Hamilton H. Anderson

Yellow Fever Vaccination, by Kenneth C. Smithburn and collaborators. World Health Organization Monograph Series No. 30, 238 pages, 37 figures. Geneva, WHO, 1956. Price $5.00; £ 1/5; Sw. fr. 15.– French edition in preparation.

Yellow fever was the first disease in man shown to be caused by a filterable virus, and research into its immunology over the past 30 years has led to the development of immunization procedures which not only have resulted in enormous social and economic benefits in the endemic area of the disease, but have served as guides to analogous advances in the whole field of virus infection. The various stages in the preparation and control of the Dakar vaccine by the Pasteur Institute at Dakar, and of the 17D vaccine by the Rockefeller Foundation in its New York Laboratories are described in detail and profusely illustrated by photographs. This is followed by a discussion of the methods and results of mass vaccination, based on the immunization of more than 50 million persons in Africa and South America. The advantages and hazards of mouse-brain and chick-embryo viruses are weighed; scarification as a technique is compared with subcutaneous inoculation; and the combination of smallpox and yellow fever immunization is discussed. There is a chapter on international regulation of yellow fever vaccination, and a select bibliography of 378 titles.

L. W. Hackett

Manual de Micologia Médica, by Carlos da Silva Lacaz, Professor de Microbiologia e Imunologia da Faculdade de Medicina da Universidade de São Paulo. Ex-Professor de Microbiologia e Imunologia da Faculdade de Medicina de Sorocaba e da Escola de Enfermagen da Universidade de São Paulo. 2nd edition. 422 pp., illustrated. São Paulo, Brazil, 1956.

This is the second edition of Professor Lacaz's excellent manual of medical mycology, amplified with new illustrations and technics, and brought up to date as regards the bibliography. In the first and more general part, the author deals with the morphology, classifi-

cation, pathology and importance of fungi of medical interest. There are special chapters on the superficial and the deep mycoses, their role in allergy and as contaminants, and laboratory methods of staining, cultivating and identifying them. A new chapter has been added on treatment and prophylaxis.

L. W. HACKETT

Précis D'Épidémiologie et Prophylaxie des Grandes Endémies Tropicales, par V. REYNES. Préface du Médecin-Général-Inspecteur Vaucel. Troisième édition revue et augmentée. 418 pp. with 57 figures. Paris, Masson et Cie., 1955. 2.800 Fr.

The appearance of a third edition shows that this book meets a need for a concise treatise in the French language on the epidemiology, prevention and control of tropical diseases. The title is misleading, however, since the text seems to be written with only the French colonial doctor in mind. For instance the distribution of *A. labranchiae* is given as North Africa, that of *A. darlingi* as French Guiana, and that of *A. minimus* as Indochina. It is in fact something more and something less than a compendium of tropical endemic diseases. On the one hand it includes many diseases not ordinarily considered to be tropical, such as pneumococcal and meningococcal infections, tuberculosis, poliomyelitis, typhoid fever, infectious hepatitis, etc., which are important enough to the colonial doctor, but which he will find, together with other common diseases of mankind, more fully described in his general medical textbooks. On the other hand, the book omits without explanation a good many diseases usually considered of tropical importance such as yaws, pinta, dengue, phlebotomus fever, the flagellate protozoal infections, coccidiosis, bartonellosis and the mycoses, some of which would certainly be encountered by the French colonial physician. The omission of nutritional disorders like kwashiorkor, sprue, pellagra and scurvy is difficult to explain, when beriberi is given an entire chapter. The book was printed at the end of 1955 but is often inaccurate or out of date; for example, *A. maculipennis* is still given as the malaria vector of Europe, and the eggs are illustrated as of one type; there is no mention of anopheline resistance to insecticides; the last important outbreak of urban yellow fever in Africa is given as that of 1927 in Dakar; African monkeys are said not to be susceptible to yellow fever virus in contrast to Asiatic and American monkeys, and so on. No selected bibliography is given for malaria, as is done for the other diseases, since the literature is said to be too vast. Names of foreign authors are frequently misspelled, e.g., Schortt, McTheiler, Taliafero, Kliger, McCallum. The illustrations are almost all borrowed from other texts—mainly from Brumpt and Manson—together with their legends, which leads to discrepancies of nomenclature in the text. The book has the qualities of brevity, clarity and consistence; it is epidemiological and not clinical. Its usefulness is limited, by its selectivity, to a rather restricted readership.

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Coatney, G. R. : Industry and Tropical Health, Volume II, 1955.

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